VOLUME 11 NOVEMBER, 1934 NUMBER 5

NATIONAL
RESEARCH COUNCIL
of CANADA

CANADIAN JOURNAL OF RESEARCH

CANADA

Published under the authority
of the
Chairman of the Committee of the
Privy Council on Scientific and Industrial Research

OTTAWA - CANADA

CONTENTS

Original Papers

Reviews and Notes

Publications and Subscriptions

The Canadian Journal of Research is published monthly by the National Research Council of Canada, Ottawa, to which address all correspondence should be directed.

The subscription rate is $3.00 per year to any part of the world. Single copies are thirty-five cents each.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 11 NOVEMBER, 1934 NUMBER 5

RAPID DETERMINATION OF MOISTURE IN GRAIN

III. CALIBRATION AND COMPARISON OF ELECTRICAL MOISTURE METERS WITH VACUUM OVEN FOR AMBER DURUM WHEAT, BARLEY AND OATS[1]

BY W. H. COOK,[2] J. W. HOPKINS[3] AND W. F. GEDDES[4]

Abstract

The previous study has been extended to include durum wheat, barley and oats. The hand-operated Tag-Heppenstall meter was found to be unsatisfactory with these grains, as they would not feed into the roller electrodes in a suitable manner. The Burton-Pitt gave erratic results with these grains and it was only possible to calibrate this meter over a limited moisture range, and even over this range it was more inaccurate than the other meters. Qualitatively the calibration curves for these three grains, in the Limbrick and motor Tag-Heppenstall, were similar to those previously obtained with hard red spring wheat. The actual resistance and the slope of the curves were, however, somewhat different for the different grains. The standard error of prediction shows that the motor-operated Tag-Heppenstall was the most accurate meter for use with durum wheat and barley, while the Limbrick was superior with oats. With the limited number of samples available it was impossible to detect any significant difference between the temperature coefficients, in any particular meter, of the different grains. When converted to a moisture basis the correction factors were practically the same as for hard red spring wheat.

The results from the entire investigation show that the Brown-Duvel method is more accurate than the 130° C. air oven method with all grains studied. The motor-operated Tag-Heppenstall meter is as accurate as the Brown-Duvel with hard red spring wheat, over the moisture range 11.0 to 17.0%, and is superior to the air oven method over this limited range. Otherwise the rapid analytical methods are more accurate than any of the moisture meters tested with any of the grains. The meters fall in the following order of decreasing accuracy over the moisture range 11.0 to 17.0%:-with hard red spring wheat; motor Tag-Heppenstall, Limbrick, hand Tag-Heppenstall, Burton-Pitt and Davies: with durum wheat and barley; motor Tag-Heppenstall, Limbrick and Burton-Pitt: and with oats; Limbrick, Burton-Pitt and motor Tag-Heppenstall. Where a meter is not mentioned no tests were made, the instrument having been omitted because it gave no promise of practical utility.

1. Introduction

The scope and plan of this investigation have already been given in earlier papers of this series (1, 2). In the present paper, the results obtained with

[1] *Manuscript received October 6, 1934.*
Contribution from the National Research Laboratories, Ottawa, and Dominion Grain Research Laboratory, Winnipeg. Published as paper No. 60 of the Associate Committee on Grain Research.
[2] *Biologist, National Research Laboratories, Ottawa.*
[3] *Statistician, National Research Laboratories, Ottawa.*
[4] *Chemist-in-charge, Dominion Grain Research Laboratory, Board of Grain Commissioners, Winnipeg.*

the Burton-Pitt, Limbrick, and hand- and motor-operated Tag-Heppenstall meters upon amber durum wheat, barley and oats, are compared with those obtained with the vacuum oven. Since the Davies meter proved quite unsuitable for hard red spring wheat (2), it was not included in the present study.

Approximately 50 samples of durum wheat and 75 of oats and of barley were employed. These were the market run of grades during the collection period but were selected with respect to moisture content so as to give a fairly uniform distribution throughout the moisture range. Since it was impossible to secure naturally damp (over 17.0% moisture) durum samples, it was necessary to prepare these. Owing to the possibility of artificially tempered grain behaving differently from naturally damp samples (2) even when allowed to stand a month after tempering, these samples were dampened by exposing them to a high relative humidity at room temperature, and then allowing them to dry down to the observed moisture content. Whether or not they behaved differently from naturally damp grain is not known as there were no naturally damp samples available for comparison.

2. Experimental Details

As in the previous experiments (2) with hard red spring wheat, the instruments were, with the exceptions noted below, operated in accordance with the manufacturers' instructions, and the sensitivities, settings and operation of the instruments were identical with those previously described. The Burton-Pitt cell would not hold 100 gm. of barley or oats, and the maximum quantity of these grains that could be employed was 80 and 60 gm. respectively. As the Limbrick cell is filled by volume no modification of the technique was required. None of these grains would feed into the rollers of the hand-operated Tag-Heppenstall meter satisfactorily at setting No. 3, which corresponded most nearly to the spacing of 40/1000 in. recommended by the manufacturers, and No. 4 and 5 were consequently tried. A few durum samples at No. 5 setting excepted, the feed was still too irregular to permit of accurate readings of the microammeter and the use of this meter with these grains was finally abandoned. The motor Tag-Heppenstall appeared to operate satisfactorily at the recommended spacing of 40/1000 in. with durum wheat and barley, but oats sometimes failed to feed through unless the undriven roller was rotated a revolution or so by hand. The microammeter readings, however, remained reasonably constant while the rolls were in operation. A roll spacing of 50/1000 in. was also tested with oats.

As before, the meter tests were made in a room conditioned as to temperature and humidity. The general reliability tests were made at 76° F., instead of 72° F. as in the hard red spring wheat studies, since it was found possible to maintain the higher temperature within narrower limits. Even with this precaution, the temperature occasionally rose 1–2° F. and the readings obtained were therefore corrected to the 76° F. basis before making

the statistical analyses. The relative humidity employed was 40%±2%, as before, and the corrections for humidity variations have already been shown in the previous experiments (2) to be too small to affect the results.

3. Effect of Temperature

Owing to the difficulty of determining accurately the temperature coefficient of the grain alone, no attempt was made to measure it with the limited number of samples available. The effect of humidity was not investigated as it has already been shown to be small, and the humidity coefficients already determined for wheat can also be regarded as applicable to these grains, since the influence of humidity must be attributed to its effect on the instruments rather than on the grain, which was kept in sealed tins until the moment of testing.

The effect of temperature was studied, using 68, 72, 76, 80, 84 and 88° F. ±½° F. and a relative humidity of 40%±2%. Four large samples of durum wheat, 10 of barley, and 12 of oats were subdivided as before for the various tests, these constituting all the large samples available for this study. The grades and moisture contents of these samples are given in Table I.

TABLE I

GRADE AND VACUUM OVEN MOISTURE CONTENT OF DURUM WHEAT, BARLEY AND OAT SAMPLES USED IN STUDYING EFFECT OF TEMPERATURE ON MOISTURE METER RESULTS

Grade*	Percentage moisture by vacuum oven						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Average
Amber durum wheat							
1 C.W. Amber durum	14.46	14.49	14.40	14.53	14.40	14.53	14.47
Tf. 2 C.W. Amber durum	16.32	16.20	16.06	16.07	15.98	16.26	16.15
Tf. 3 C.W. Amber durum	16.19	16.10	16.03	—	15.98	16.20	16.10
Tf. 2 C.W. Amber durum	16.93	17.06	16.92	17.03	16.73	16.78	16.91
Barley							
3 Ex. C.W. Six-row	14.41	14.34	14.46	14.45	14.40	14.40	14.41
3 Ex. C.W. Six-row	13.72	13.28	13.34	13.32	13.28	13.30	13.37
3 Ex. C.W. Six-row	14.66	14.59	14.70	14.56	—	14.62	14.63
Tf. 3 C.W.	17.14	17.00	17.00	17.10	16.91	17.00	17.03
Tf. 3 C.W.	14.92	14.96	14.78	14.94	14.75	14.90	14.88
Tf. 3 C.W.	16.54	16.48	16.34	16.50	16.46	16.48	16.47
Tf. 3 Ex. C.W. Six-row	17.72	17.80	17.78	17.78	17.76	17.66	17.75
Dp. 3 C.W.	22.04	21.76	21.70	21.82	21.82	21.90	21.84
Dp. 3 C.W.	—	19.64	19.56	19.66	19.52	19.76	19.63
Dp. 3 Ex. C.W. Two-row	22.35	22.37	22.12	22.25	22.20	22.44	22.29
Oats							
3 C.W.	13.11	13.10	13.26	13.06	13.12	13.08	13.12
3 C.W.	14.39	14.52	14.40	14.35	14.42	14.37	14.41
1 Feed	14.57	14.61	14.79	14.66	14.60	14.60	14.64
Tf. 2 C.W.	15.34	15.32	15.42	15.28	15.32	15.26	15.32
Tf. 2 Feed	17.45	17.37	17.54	17.20	17.47	17.44	17.41
Dp. 3 C.W.	17.62	17.63	17.72	17.56	17.73	17.62	17.65
Dp. 1 Feed	17.60	17.57	17.76	17.54	17.57	17.56	17.60
Dp. 3 C.W.	19.61	19.62	19.68	19.57	19.68	19.64	19.63
Dp. 3 C.W.	18.04	17.86	18.14	17.11	17.98	18.12	17.88
Dp. 1 Feed	19.30	19.30	18.69	19.25	19.30	19.34	19.20
Dp. Ex. 1 Feed	22.07	22.08	22.08	22.10	22.06	21.94	22.06
Dp. Ex. 1 Feed	22.48	22.55	22.60	22.51	22.59	22.54	22.55

* *Tf.* = *tough.* *Dp.* = *damp.*

The results obtained with the three meters are plotted in Fig. 1 for both durum wheat and barley, and in Fig. 2 for oats. With the Burton-Pitt, the meter readings are plotted against the temperature, but with the resistance type instruments the logarithm of the resistance is employed since this quantity is proportional to the moisture content over the greater part of the moisture range. As the resistances measured by the Limbrick were much higher than those encountered in the Tag-Heppenstall, the megohm and 1000 ohms respectively were selected as units of resistance.

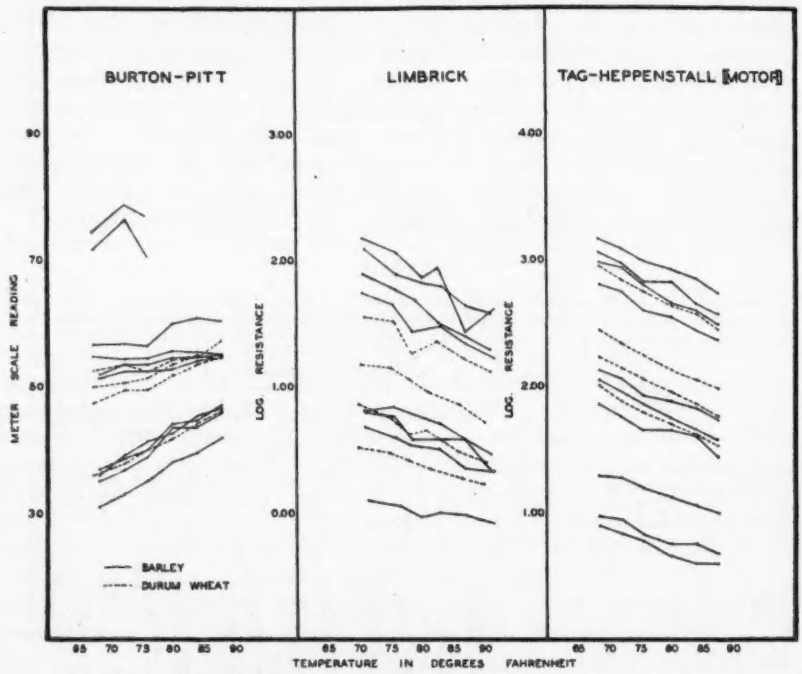


FIG. 1. *Effect of temperature of grain-instrument system on meter reading. (Durum wheat and barley).*

It is evident from Figs. 1 and 2 that the Burton-Pitt gave erratic results at deflections greater than about 55 scale divisions, as it did with hard red spring wheat. The variability observed with any one sample in the other two meters can doubtless be attributed, as before, to experimental and sampling errors.

In order to determine the temperature coefficient, a straight line was fitted to the points obtained with each sample, by the method of Least Squares. The equations were then differentiated to obtain the temperature coefficients, which are plotted against the observed meter reading or logarithm of the resistance at 76° F. (79° F. for the Limbrick) in Fig. 3. With the limited number of samples tested, it was impossible to demonstrate any difference

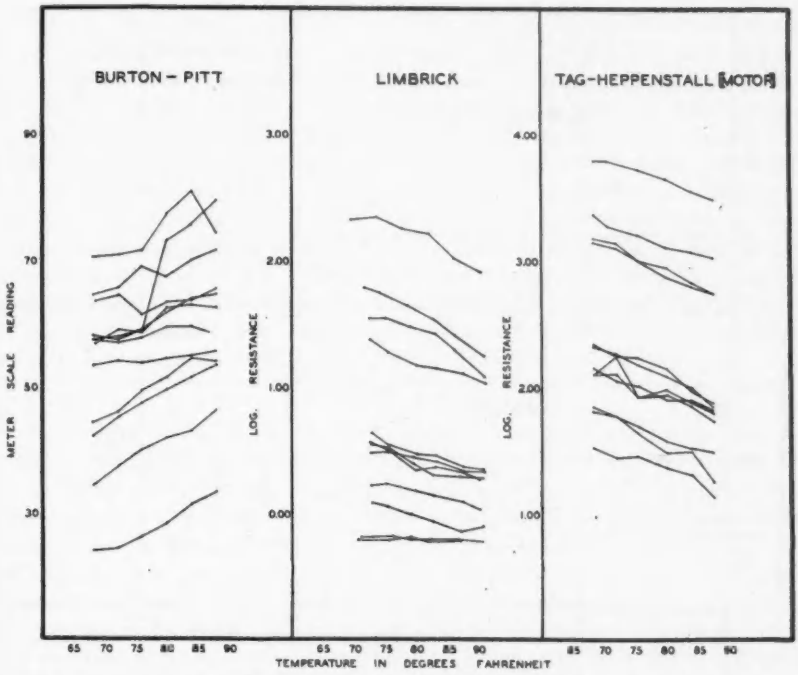


FIG. 2. *Effect of temperature of grain-instrument system on meter reading. (Oats).*

TABLE II

TEMPERATURE COEFFICIENT OF GRAIN-INSTRUMENT SYSTEM
(Grain and meter at same temperature)

Meter	Meter range	Temp. coefficient per ° F.	Standard error	Unit of measurement
Burton-Pitt	20 – 70	0.30	0.05	Meter scale division
Limbrick	over 1.00	−0.0248	0.0015	Logarithm of resistance in megohms
	1.00 – 0.50	−0.0168	0.0014	
	0.50 – 0.00	−0.0144	0.0009	
	under 0.00–	−0.0056	0.0028	
Tag-Heppenstall (motor)	4.00 – 0.50	−0.0214	0.0007	Logarithm of resistance in 1000's of ohms

between the temperature coefficients for the three grains. It is evident that the Limbrick meter is the only one in which there is a systematic variation in the coefficient with observed resistance (moisture content), the Burton-Pitt instrument giving erratic results, and the coefficients for the motor Tag-

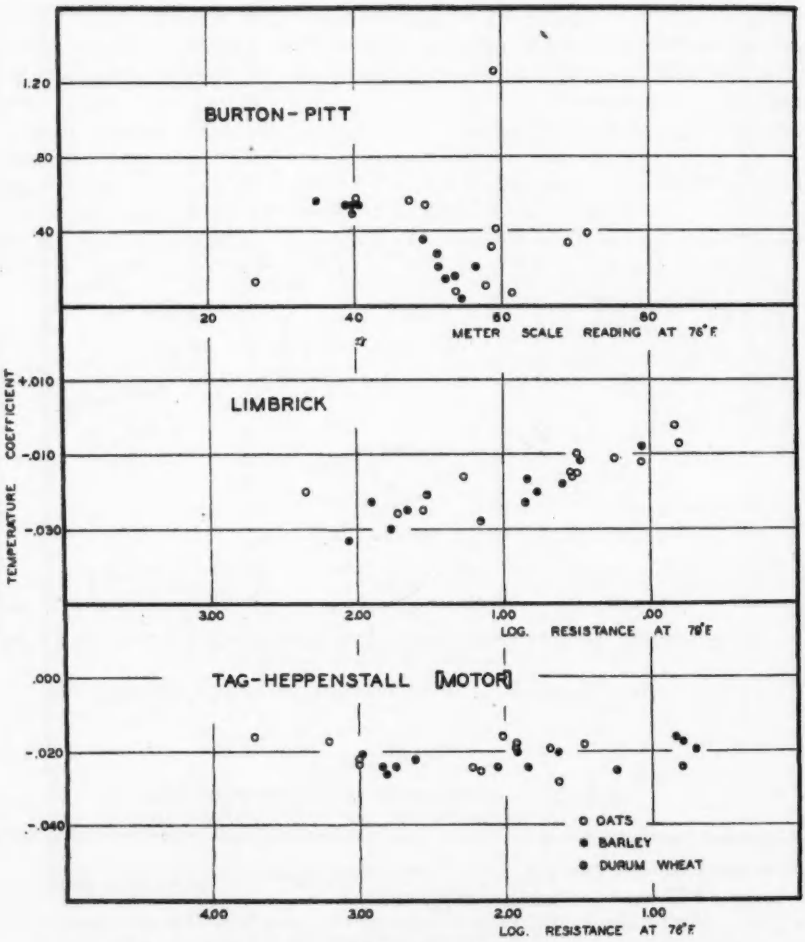


FIG. 3. *Variation of temperature coefficient of grain-instrument system with moisture content. (Durum wheat, barley and oats).*

Heppenstall being practically the same throughout the range. The values used for correcting the results obtained in the general reliability series for small variations in temperature are given in Table II. Owing to the variability of the coefficients obtained with the Burton-Pitt an average of all the coefficients was used.

4. Calibration and Accuracy of Meters

The meters were tested in a conditioned room as before and the readings were corrected for the small temperature fluctuations which occurred. A

standard temperature of 76° F. was used for the Burton-Pitt and motor Tag-Heppenstall meters, and a temperature of 79° F. for the Limbrick to allow for the heating of the grain in the preparatory grinding.

The calculations necessary to calibrate the meters with all three grains, and to determine the degree of accuracy attained, were made in exactly the same manner as those already described in the study with hard red spring wheat. Qualitatively, the calibration curves were all found to be similar to those previously obtained with hard red spring wheat, the resistance instruments indicating a linear relation between moisture content and logarithmic resistance up to about 17.0% moisture, and a curvilinear relation at higher moisture contents, where successive increments of moisture resulted in a progressively smaller diminution in resistance. Quantitatively, however, some differences were exhibited by the various grains.

Burton-Pitt Tester

Apart from reduction of the weight of barley and oats to an amount which the test-cell would accommodate, these experiments were conducted at the same sensitivity and in the same manner as those with hard red spring wheat already described. The data are shown in Fig. 4 where the moisture content by the vacuum oven is plotted against the meter scale reading. It is evident from the scattered points that the meter is not capable of great precision with any of the three grains even at low moisture content, and at high moisture contents it gives exceedingly erratic results. In consequence, curves were fitted over the lower moisture range only, and the upper limit of this range varied with the different grains according to the quality of the results. Within the range of calibration the curves are all of the same type as those obtained with hard red spring wheat, namely, parabolic with a tendency toward greater deflections with successive increments of moisture.

The experimental error, calibration equations, and errors of prediction applicable to the different grains over the range covered by the curves, are given in Table III. The experimental errors are practically the same, in all

TABLE III

EXPERIMENTAL ERROR, CALIBRATION EQUATIONS, AND OBSERVED AND NET STANDARD ERROR OF PREDICTION OF VACUUM OVEN MOISTURE CONTENT OF DURUM WHEAT, BARLEY AND OATS BY THE BURTON-PITT MOISTURE TESTER

Grain	Moisture range, %	Experimental error (standard error of duplicates), moisture %	Calibration equation*	Standard error of prediction	
				Observed moisture %	Net, moisture %
Amber durum wheat	11.5 – 18.5	0.06	$M_V = 7.56 + 0.199x - 0.000718x^2$	0.46	0.44
Barley	11.0 – 16.0	0.09	$M_V = 4.42 + 0.401x - 0.00340x^2$	0.84	0.83
Oats	12.5 – 15.5	0.05	$M_V = 8.23 + 0.244x - 0.00186x^2$	0.41	0.39

Standard errors apply to mean of duplicates.

*M_V = moisture content as determined by vacuum oven.
x = meter scale reading.*

cases, as those obtained with hard red spring wheat. The errors of prediction increase in the order: oats, durum wheat and barley, but, since the calibrated range was different in each case and was more or less arbitrarily determined,

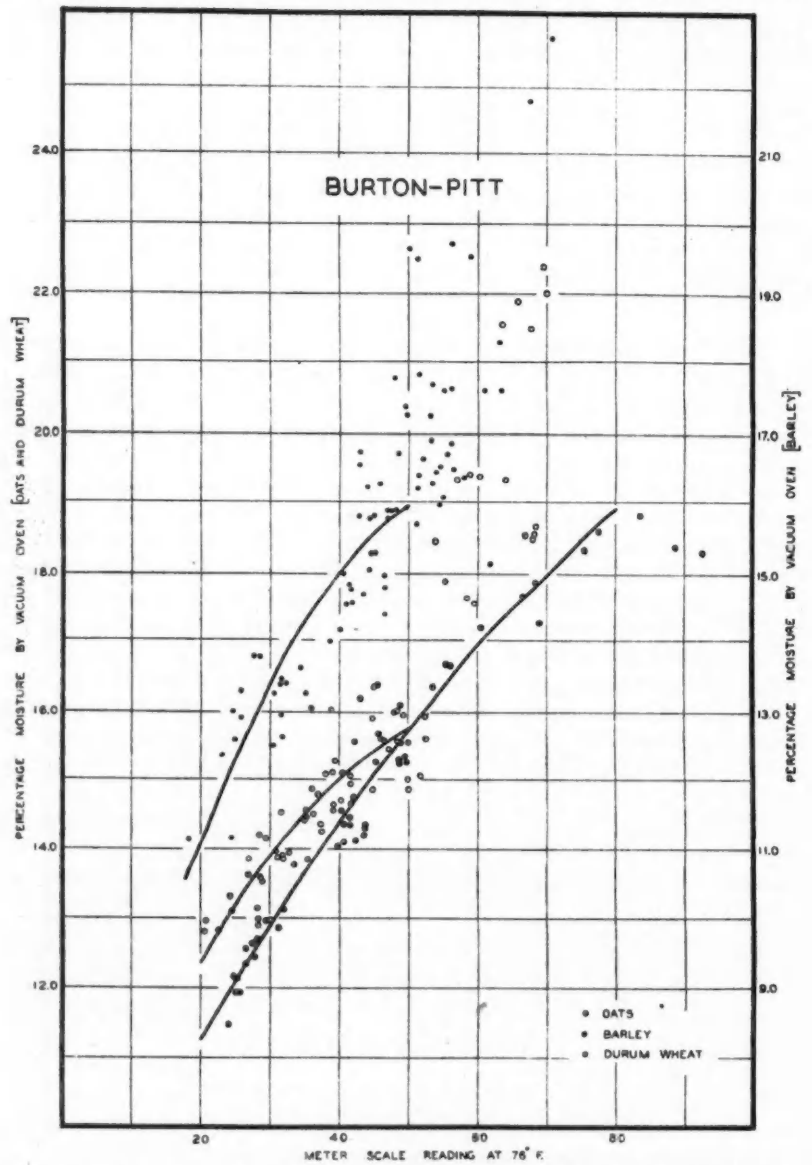


FIG. 4. *Relation between moisture content of durum wheat, barley and oats, and Burton-Pitt meter reading.*

the curves are not strictly comparable. Examination of Fig. 4 shows that, in general, this meter is less accurate with oats and barley than with durum wheat. Since the lowest error of prediction obtained, even in the lower moisture range, was 0.39% moisture, it is obvious that this meter is incapable of giving a precise estimate of the moisture content of any of the grains tested.

Limbrick Moisture Tester

The moisture content of the samples of all three grains is plotted against the logarithm of the resistance, in megohms, in the Limbrick at 79° F. in Fig. 5. Two curves were fitted to the points for each grain, one representing the linear relationship at moisture contents below about 17.0% and the other the parabolic relationship at higher moisture contents, where successive increments of moisture result in a progressively smaller diminution in resistance. Owing to the relatively small number of samples, the extremes of the two curves do not coincide. In such instances a dotted tangent indicates the probable approximate course of the actual calibration curve.

In this meter, for which the grain is ground, the two covered grains yield practically identical calibration curves, although the individual results with oats are the more erratic. Durum wheat on the other hand yields a significantly different calibration, a given change in moisture content causing a greater change in resistance. This resembles more closely the calibration deduced for hard red spring wheat. Statistical comparisons were made between the calibration equations in the "straight" and "tough" moisture range for hard red spring wheat and durum wheat, and for barley and oats. It was found that the average resistance of durum wheat was lower than that of hard red spring wheat, and also that the change in resistance per unit change in moisture was slightly greater. Similar calculations showed that oats had a slightly higher resistance than barley at a given moisture content, but that there was no significant difference in the slope of the two calibration curves. It is probable that the higher resistance of oats can be attributed to the smaller amount employed, the weight per unit volume of ground oats being less than that of ground barley.

The experimental error, calibration equations and errors of prediction applicable to each grain are given in Table IV. The experimental errors with durum wheat and barley are somewhat smaller than those encountered with hard red spring wheat. That with oats, however, is somewhat larger, especially in the "damp" range. This large experimental error can doubtless be attributed to the light chaffy character of ground oats. It is difficult to fill and strike off the small measuring cup precisely with such material, and in addition the resistance reading is dependent on the material falling between the prongs and walls of the test cell in a uniform manner in different trials, a condition which probably is not attained to the same extent with oats as with the other grains. In spite of this large experimental error, however, the error of prediction with oats is of the same order as that obtained with wheat. This suggests that although the meter is not particularly accurate in measuring

the resistance of ground oats, the relationship between resistance and moisture content is the predominating factor governing the accuracy of the meter.

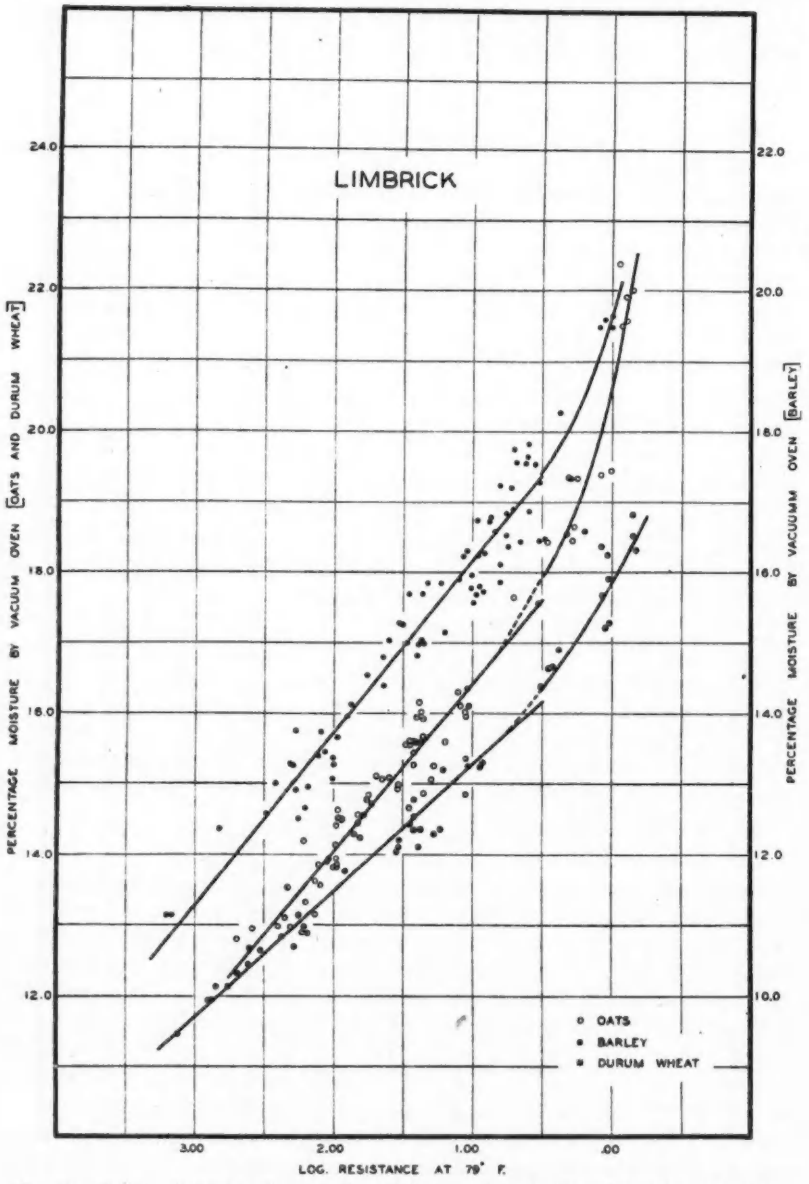


FIG. 5. *Relation between moisture content of durum wheat, barley and oats, and logarithm of resistance in megohms. Limbrick meter.*

It is evident from the error of prediction that with these grains, as with hard red spring wheat, this meter is, in general, capable of greater accuracy in the moisture range below, than in the moisture range above 17.0%. In this lower moisture range it is less accurate with oats than with durum wheat and is still less accurate with barley. Above 17.0% moisture this meter is somewhat less accurate with oats than with barley.

TABLE IV

EXPERIMENTAL ERROR, CALIBRATION EQUATIONS, AND OBSERVED AND NET STANDARD ERROR OF PREDICTION OF VACUUM OVEN MOISTURE CONTENT OF DURUM WHEAT, BARLEY AND OATS BY THE LIMBRICK MOISTURE TESTER

Grain	Moisture range, %	Experimental error (standard error of duplicates), moisture %	Calibration equation*	Standard error of prediction	
				Observed, moisture %	Net, moisture %
Amber durum wheat	11.5–16.0	0.04	$My = 17.07 - 1.79 \log R$	0.33	0.29
	16.0–19.0	0.05	$My = 17.84 - 3.52 \log R + 1.16 (\log R)^2$	0.34	0.30
Barley	11.0–17.5	0.07	$My = 18.61 - 2.44 \log R$	0.44	0.42
	17.5–20.0	0.06	$My = 19.65 - 6.62 \log R + 4.28 (\log R)^2$	0.52	0.50
Oats	12.5–17.0	0.12	$My = 18.78 - 2.38 \log R$	0.32	0.30
	17.0–22.0	0.19	$My = 20.74 - 8.88 \log R + 6.56 (\log R)^2$	0.58	0.57

Standard errors apply to mean of duplicates.
*My = *moisture content as determined by vacuum oven.*
R = *resistance in megohms.*

MOTOR-OPERATED TAG-HEPPENSTALL METER

This Tag-Heppenstall meter was operated at a roll spacing of 40/1000 in. with all three grains, as specified by the manufacturers. It was found, however, that oats did not feed into the rollers satisfactorily at this spacing, the undriven roller frequently stopping, requiring to be rotated through a revolution or two by hand. Furthermore, the microammeter needle seemed to fluctuate more than in the tests with other grains. A roll spacing of 50/1000 in. was also tested, but this gave no significant increase in accuracy.

In Fig. 6, the moisture content of the samples of all three grains is plotted against the logarithm of the resistance in 1000's of ohms, at a temperature of 76° F. and a roll spacing of 40/1000 in. As before, two curves were fitted to the points for each grain, one representing the linear relationship at moisture contents below about 17.5%, and the other the parabolic relationship at higher moisture contents.

In this meter, for which the grain is not ground, the change in resistance per unit change in moisture is again practically the same for both oats and barley, but the actual resistance at a given moisture content is much higher for oats. In the Limbrick, for which the grain is ground, oats exhibited only a slightly higher resistance than barley. This suggests that the resistance of

one of these grains is altered appreciably by grinding, but since the resistances of the whole and ground material were determined in two different instruments

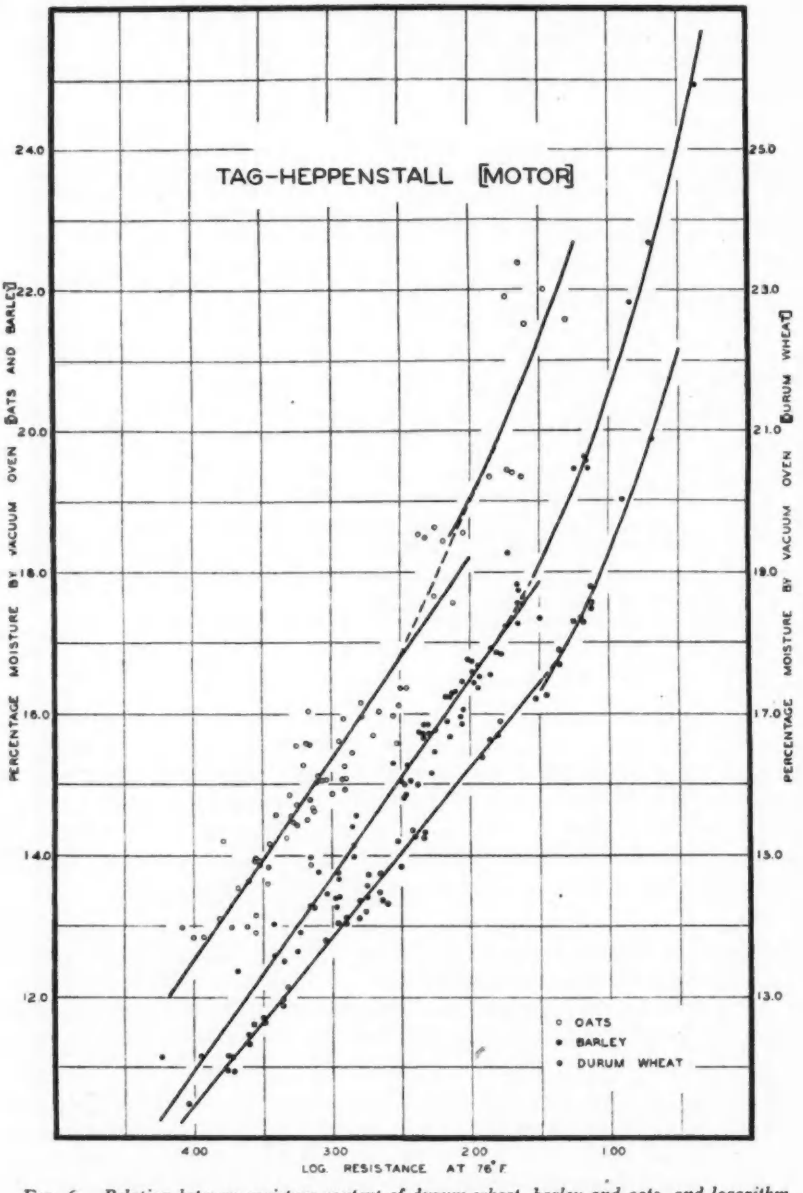


FIG. 6. *Relation between moisture content of durum wheat, barley and oats, and logarithm of resistance in 1000's of ohms. Motor-operated Tag-Heppenstall meter.*

it cannot be said which one was affected. On the other hand this apparent change in the resistance may be attributable to some peculiarity of the instruments, especially since difficulty was experienced in feeding oats into the roller electrodes of the Tag-Heppenstall meter satisfactorily. The calibration curves for oats and barley did not differ significantly in slope over the moisture range 11.0 to 17.0%.

It is evident from Fig. 6 that the durum wheat shows a slightly greater change in resistance, for a given change in moisture content, than the two covered grains. Comparisons were made between the slope and actual resistances of the calibration curves obtained with durum and hard red spring wheat. It was found that durum wheat has a significantly lower resistance than hard red spring wheat, but the difference in the slopes did not significantly exceed the standard error. These two wheats, although differing in actual resistance, seem to show the same relationship between increments of moisture and resistance, a fact which may be helpful in preparing calibration charts for them.

The experimental error, calibration equations, and errors of prediction applicable to each grain are given in Table V. The experimental error with durum wheat is practically the same as that previously obtained with hard red spring wheat, somewhat larger with barley, and still larger with oats. The standard error of prediction is least with durum wheat, intermediate with barley, and extremely high with oats, this meter being inferior to the Limbrick with this grain. This result cannot be attributed to the experimental errors, for although these fall in the same order for the different grains, they are in all cases small compared with the error of prediction. The magnitude of the prediction errors shows definitely that the instrument

TABLE V

EXPERIMENTAL ERROR, CALIBRATION EQUATIONS, AND OBSERVED AND NET STANDARD ERROR OF PREDICTION OF VACUUM OVEN MOISTURE CONTENT OF DURUM WHEAT, BARLEY AND OATS BY THE MOTOR-OPERATED TAG-HEPPENSTALL MOISTURE METER

Grain	Moisture range, %	Experimental error (standard error of duplicates), moisture %	Calibration equation*	Standard error of prediction	
				Observed, moisture %	Net, moisture %
Amber durum wheat	11.5 – 17.5	0.02	$M_V = 21.00 - 2.38 \log R$	0.20	0.13
	17.5 – 22.0	0.04	$M_V = 26.05 - 8.81 \log R + 1.99 (\log R)^2$	0.18	0.10
Barley	11.0 – 17.5	0.04	$M_V = 21.96 - 2.75 \log R$	0.32	0.29
	17.5 – 25.0	0.06	$M_V = 28.66 - 10.38 \log R + 2.24 (\log R)^2$	0.37	0.34
Oats	12.5 – 17.5	0.07	$M_V = 23.90 - 2.85 \log R$	0.56	0.55
	17.5 – 22.5	0.13	$M_V = 32.58 - 9.84 \log R + 1.55 (\log R)^2$	1.01	1.00

Standard errors apply to mean of duplicates.

*M_V = *moisture content as determined by vacuum oven.*

R = *resistance in 1000's of ohms.*

is much less reliable with the covered grains than with wheat. It seems probable that the error of prediction could be lowered by grinding the covered grains, especially oats, since the Limbrick, for which the grain is ground, gave a lower error of prediction with oats even though the experimental error was relatively high.

Day-to-day Variations

As explained in the previous paper (2) three large samples of each of the grains were tested daily in all meters to determine to what extent the results were affected by daily fluctuations. Owing to the small number of samples included in the general reliability series, the test with any one grain usually did not extend over more than two or three days, and the results obtained from the above large samples showed no evidence of daily fluctuations within this limited period. Calculation of the variance and covariance of the results in the general reliability tests, around the daily means as well as around the general means, likewise gave no evidence of daily variations, except to a slight extent in the barley experiments with the Burton-Pitt.

5. Temperature Corrections in Terms of Moisture

The temperature coefficients, given earlier in Table II, were obtained by pooling the results from the limited number of samples of the individual grains, and while adequate for correcting the results of the general reliability tests for the small variations which occurred, are not recommended for general use. They are, however, judged to be sufficiently accurate for "straight" and "tough" samples of oats and barley in the two resistance instruments to permit temperature corrections to be made over a range of ±10° F. Correction values, showing the percentage moisture to be added or subtracted for each degree F. above or below the temperature at which the calibration curve is applicable were therefore computed for these grains and meters as previously described (2) and are

TABLE VI

Temperature corrections in terms of per cent moisture
(Grain and meter at same temperature)

Meter	Standard temperature, °F.	Correction in terms of per cent moisture for each degree F. above or below the standard temperature	
		Straight	Tough
Barley			
Limbrick	79	0.06	0.05
Tag-Heppenstall (motor)	76	0.06	0.06
Oats			
Limbrick	79	0.06	0.05
Tag-Heppenstall (motor)	76	0.06	0.06

Note: *Corrections are to be* added *if the temperature is below the standard, and* subtracted *if above the standard.*

listed in Table VI. The corrections for the two meters differ slightly, but the corresponding values are practically the same as those for hard red spring wheat (*cf.* (2) Table X). This suggests that the same correction may be applicable to all grains.

7. General Summary and Conclusions

As a matter of convenience, a brief summary of the results obtained from the entire investigation is given here. The result of an analysis of variance on the vacuum oven data has already been summarized in the first paper of this series ((1) Table III). The sampling error proved to be the greatest single source of variation. Day-to-day variations in the results also occurred, and the two vacuum ovens, although of identical construction, were found to give results differing on the average by 0.07% moisture. It is probable that the average differences between ovens and the day-to-day variations are to some extent different measurements of the same effect.

The results obtained with the 130° C. air oven, and the Brown-Duvel have also been summarized ((1) Table VIII). It is evident from these that the Wiley mill was superior to the Hobart grinder for use in conjunction with the air oven method. The Brown-Duvel method proved, under the conditions of these experiments, to be superior to the 130° C. air oven, even when the Wiley mill was employed. Both methods, however, underestimate the moisture content of all the grains studied, but this systematic error may be eliminated by means of the linear correction equations given in the first paper ((1) Tables IV, VI and VII).

The experimental error, and the net standard error of prediction of vacuum oven moisture content for all meters calibrated and all grains tested, are given in Table VII. It is evident that the experimental error, as in the rapid analytical methods, is in all cases small compared with the standard error of prediction, indicating that the meters are capable of measuring the resistance, or other electrical quantity, accurately enough, but that this is affected by some physical property of the grain other than moisture content. This property may be inherent in the grain or it may be induced by the instrument, *e.g.*, by polarization. The meters are arranged in the table in order of decreasing accuracy, as determined by the standard error of prediction in the moisture range 11.0 to 17.0%, which is most important commercially, for each of the grains tested. It is evident that the motor-operated Tag-Heppenstall is the most accurate of the meters for all grains excepting oats, for which the Limbrick proved to be best, possibly because it employs ground grain. The Burton-Pitt and Davies instruments are judged of little practical value.

The results by all meters were found to be appreciably affected by fluctuations in temperature, and the temperature coefficient of wheat alone was found to differ appreciably from that of the grain-instrument system in the Burton-Pitt and Limbrick testers, as shown in the second paper of the series ((2) Table X), suggesting a significant instrumental temperature coefficient.

The meters were also all influenced to some extent by humidity conditions, the effect being greatest in the Limbrick. In general, however, a 10% change in relative humidity at 72° F. had less effect than a 1° F. change in temperature.

TABLE VII

"EXPERIMENTAL ERROR" AND NET STANDARD ERROR OF PREDICTION OF VACUUM OVEN PERCENTAGE MOISTURE BY ELECTRICAL MOISTURE METERS

Meter	Moisture range, %	Experimental error (standard error of duplicates), moisture %	Net standard error of prediction, moisture %
	Hard red spring wheat		
Tag-Heppenstall (motor)	11.0 to 17.0	0.02	0.16
	17.0 to 24.0	0.03	0.56
Limbrick	11.5 to 17.0	0.08	0.38
	17.0 to 21.5	0.09	0.49
Tag-Heppenstall (hand)	11.0 to 17.5	0.04	0.40
	17.0 to 24.0	0.05	0.44
Burton-Pitt	11.0 to 17.0	0.06	0.54
	Amber durum wheat		
Tag-Heppenstall (motor)	11.5 to 17.5	0.02	0.13
	17.5 to 22.0	0.04	0.10
Limbrick	11.5 to 16.0	0.04	0.29
	16.0 to 19.0	0.05	0.30
Burton-Pitt	11.5 to 18.5	0.06	0.44
	Barley		
Tag-Heppenstall (motor)	11.5 to 17.5	0.04	0.29
	17.5 to 25.0	0.06	0.34
Limbrick	11.0 to 17.5	0.07	0.42
	17.5 to 20.0	0.06	0.50
Burton-Pitt	11.0 to 16.0	0.09	0.83
	Oats		
Limbrick	12.5 to 17.0	0.12	0.30
	17.0 to 22.0	0.19	0.57
Burton-Pitt	12.5 to 15.5	0.05	0.39
Tag-Heppenstall (motor)	12.5 to 17.5	0.07	0.55
	17.5 to 22.5	0.13	1.00

Standard errors apply to mean of duplicates.

Comparison of the results obtained by the rapid analytical methods and moisture meters shows that the Brown-Duvel is as accurate with hard red spring wheat throughout the entire moisture range tested as the motor-operated Tag-Heppenstall is over the limited range 11.0 to 17.0%. At higher moisture contents, or with any of the other grains examined, the Brown-Duvel method is superior in accuracy to any of the electrical testers. The 130° C. air oven method, using a Wiley mill for grinding, is also more accurate than any of the meters, except the motor-operated Tag-Heppenstall, in the lower moisture range with hard red spring wheat.

Acknowledgments

The authors wish again to express their appreciation of the able assistance rendered by the individuals referred to in the first two papers of this series. They also wish to thank the various manufacturers who loaned moisture meters for study.

References

1. Cook, W. H., Hopkins, J. W. and Geddes, W. F. Rapid determination of moisture in grain. I. Comparison of 130° C. air oven and Brown-Duvel methods with vacuum oven method. Can. J. Research, 11 : 264-289. 1934.

2. Cook, W. H., Hopkins, J. W. and Geddes. W. F. Rapid determination of moisture in grain. II. Calibration and comparison of electrical moisture meters with vacuum oven for hard red spring wheat. Can. J. Research, 11 : 409-447. 1934.

STUDIES ON THE NATURE OF DISEASE RESISTANCE IN CEREALS[1]

I. THE REACTIONS TO RUST OF MATURE AND IMMATURE TISSUES

By Margaret Newton[2] and A. M. Brown[3]

Abstract

A study has been made of the reactions of mature and immature tissues of wheat, oats, barley and rye to different cereal rusts. For a short time before cereal plants come into head, there are parts in the upper half of the culm, *e.g.*, the basal portion of the peduncle and of the flag-leaf sheath, that are growing and elongating very rapidly and are thus composed of soft succulent tissue. Growth in the remainder of the culm is proceeding at a much slower rate and hence in these parts most of the tissue is relatively firm and mature. In varieties and strains of wheat, oats and barley which, when inoculated in the seedling stage in the usual way with *Puccinia graminis*, produce a "1" type of pustule, it has been found that, if the different parts of the culm are inoculated by injection with a suspension of urediospores shortly before the plants come into head, the young, rapidly growing parts are very susceptible while the older, more mature parts are highly resistant. A similar result is obtained with plants possessing mature plant resistance. Both resistant and susceptible tissues are therefore present in the same plant at the same time. The young, rapidly growing parts are also susceptible to a number of rusts which are not natural parasites of the particular cereal: *P. graminis tritici* will attack oats and rye, and *P. graminis avenae* and *P. triticina* will attack barley. As the susceptible parts grow older they become as resistant as the rest of the plant. During their susceptible period they are generally quite effectively protected from infection by plant parts that are resistant.

Introduction

It is now well known that some wheat varieties possess a high degree of resistance to stem rust (*Puccinia graminis tritici* Erikss. and Henn.), but it is not yet clearly understood why such varieties are resistant. The same may be said generally regarding cereals and their rusts. A number of investigators (6, 7, 12, 13, 14, 18, 19, 20) have concerned themselves with the problem of the nature of resistance to stem rust, and considerable information has been accumulated regarding the morphology, physiology and chemistry of resistant and susceptible wheat varieties. In general the method followed has been to compare, with respect to morphological, physiological, and chemical aspects, the tissues of resistant and susceptible varieties. As the varieties compared may differ from one another in many other respects than their reaction to rust, certain disadvantages are inherent in such a procedure. If both susceptible and resistant tissues could be found at one and the same time in plants of any particular variety, there would be an obvious advantage in using such a variety for the studies.

In 1930, Goulden, Newton and Brown (11) compared the reactions of 14 wheat varieties at two stages of maturity to 16 forms of *P. graminis tritici* and discovered that in some of these varieties the adult plants possess both susceptible and resistant tissues at the same time. Marquis and Quality

[1] *Manuscript received July 27, 1934.*
Contribution No. 407 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.
[2] *Senior Plant Pathologist, Dominion Rust Research Laboratory, Winnipeg, Canada.*
[3] *Assistant Plant Pathologist, Dominion Rust Research Laboratory, Winnipeg, Canada.*

were found to be resistant in the seedling stage to forms 38 and 53, and were considered resistant also in the adult stage to these forms; but, when plants of these varieties were growing rapidly in the adult stage, pustules of the "4" type developed on them in the region just above the nodes and on that portion of the neck (peduncle) which last emerged from the sheath, although the remaining portions of the plants, similarly inoculated, were quite resistant. As adult plants of Marquis and Quality were found to be susceptible in certain parts and resistant in other parts to these two forms of stem rust the varieties were said to possess "regional resistance".

The finding of susceptible and resistant regions in the same plants of each of these two varieties suggested that a similar condition might be found in some other varieties of wheat, and possibly also in varieties of oats, rye and barley. If susceptible and resistant regions were found to be present in plants of a number of different varieties, these varieties would provide favorable material for investigations by biochemists and plant physiologists on the nature of rust resistance. It seemed desirable therefore to ascertain to what extent this phenomenon is present among varieties of cereals. The present paper presents the results thus far obtained in a study of this phase of the problem, and includes the results reported earlier elsewhere in abstract form (18).

Experimental Methods

As field experiments by the senior author had shown that heavier infections were usually secured when the inoculum was deposited within the tissues than when it was applied in the ordinary manner to the exterior of the plant, in all the experiments here reported the plants were inoculated according to the method suggested by Zehner and Humphrey (27), *i.e.*, by injecting a distilled-water suspension of urediospores into the plant tissue by means of a hypodermic syringe.

Each internode was inoculated at a point about one inch above the node, the inoculations being made while the plants were still growing vigorously and usually when the heads (enclosed in the sheaths) were only partially developed. During the process of inoculation the syringe was held almost parallel to the plant, the needle entering the tissue at a very sharp angle. At each inoculation the needle passed through two leaf-sheaths, sometimes through three when the inoculation was made above the uppermost node. Thus the inoculum was forced in between the sheaths, and between the sheaths and the culms or heads. Only a small amount of it was lost through leakage from the puncture. Repeated experiments demonstrated that when a small amount of the leaf suspension oozed from the top of the sheaths, the tissue involved was satisfactorily inoculated. It was found more difficult to inoculate the older or lower part of a plant than the younger or upper part. No attempt was made to inject the inoculum into the individual leaves, but the flag leaf, if still enclosed, was automatically inoculated by the suspension which was forced up inside the sheath enveloping this leaf. Immediately after inoculation, the plants were placed on benches in the greenhouses where

they were left until the time arrived for recording the results. All the experiments were carried out in two different sections of the greenhouse, kept at temperatures of 60° F. and 75° F. respectively. About three weeks were required for full rust development in the cooler section, and about two weeks in the warmer section.

The symbols used in recording the types of infection on the different hosts are those originally employed by Stakman and Levine (25) and have the following significance:—

Type 0. Host immune. No uredinia are formed; hypersensitive flecks are frequently present. The reaction is then indicated by ";".

Type 1. Host very resistant. Uredinia are minute, each being surrounded by a well defined necrotic area.

Type 2. Host moderately resistant. Uredinia are small to medium in size, and are commonly found in islands of green tissue surrounded by necrotic halos.

Type 3. Host moderately susceptible. Uredinia are medium in size, and do not coalesce very frequently. Hypersensitiveness is absent, but the pustules may be surrounded by chlorotic areas.

Type 4. Host very susceptible. Uredinia are large and generally confluent. Hypersensitiveness is absent, but slight chlorosis may accompany pustules.

Type x. Host reaction heterogeneous. Uredinia on the same leaf vary in size; frequently all the types of infection are found occurring together on the same leaf.

The signs (=), (−), (±), (+) are used to indicate quantitative variations in the above types.

Regional Susceptibility in Adult Plants of Wheat, Oat and Barley Varieties Possessing Seedling Resistance

Wheat Varieties

For these experiments, wheat varieties and strains were selected which, in the seedling stage, were known to possess resistance to certain physiologic forms. These wheats included (i) those which are immune in the seedling stage, such as Marquis × Kanred to form 21, and Iumillo to form 36; (ii) those which are highly resistant in the seedling stage, such as Khapli to forms 21 and 36, Kota to forms 2 and 38, Marquis to form 38, and Speltz Marz to form 36; (iii) those which are semi-resistant in the seedling stage (*i.e.*, develop an "x" type of infection), such as Marquis to form 120, and Speltz Marz to form 38.

It should, however, be pointed out that the reactions just referred to are those that arise from the application of spores to the exterior of plants, the ordinary routine method of inoculation. Previous experiments with resistant varieties had shown that the two methods of inoculation did not appear to result in the same type of infection.

TABLE I

HOSTS TESTED FOR INDICATIONS OF REGIONAL SUSCEPTIBILITY

Species	Variety	Source
Triticum		
T. compactum	Little Club	R.L.*223, C.I. 4066
T. dicoccum	Khapli	R.L. 563, C.I. 4013
T. durum	Acme	R.L. 566, C.I. 5284
T. durum	Iumillo	R.L. 7
T. durum	Pentad	R.L. 203
T. durum	Speltz Marz	R.L. 569, C.I. 6236
T. vulgare	Chul	R.L. 543
T. vulgare	Garnet	R.L. 15
T. vulgare	Hope	R.L. 209
T. vulgare	H-44-24	R.L. 229
T. vulgare	Kota	R.L. 571, C.I. 5878
T. vulgare	Marquis	R.L. 572, C.I. 3641
T. vulgare	Marquis × Kanred	R.L. 226
Avena		
A. sativa	Anthony	R.L. 1075
A. sativa	Green Russian	R.L. 371
A. sativa	Joanette	R.L. 561
A. sativa	Richland	R.L. 172, C.I. 787
A. sativa	Ruakura	R.L. 345, C.I. 2025
A. sativa	Victory	R.L. 159
A. sativa orientalis	Green Mountain	R.L. 929, C.I. 1892
A. sativa orientalis	White Russian	R.L. 177, C.I. 551
A. sterilis	Belar	C.I. 2760
A. sterilis	Red Rustproof	C.I. 1815
A. sterilis	Sterisel	C.I. 2991
A. sterilis	Sunrise	C.I. 982
Hordeum		
H. vulgare	Manchurian	Minnesota Selection 184
H. vulgare	O.A.C. 21	C.I. 1470
H. vulgare	Peatland	R.L. 656
H. vulgare	Success	C.I. 1808
Secale		
S. cereale	Colorless	Minnesota No. 104
S. cereale	Rosen	Minnesota Accession No. 82
S. cereale	Swedish	Minnesota No. 2

* *R.L. indicates the accession numbers of the plant breeding section of the Dominion Rust Research Laboratory.*

A number of adult plants of other varieties were tested to different forms to which they are wholly susceptible in the seedling stage, but as all parts of such plants became almost equally heavily infected, the varieties were considered useless as indicators of regional susceptibility and were not included in further tests.

From the results presented in Table II, it appears that the method employed in the inoculation of a resistant adult plant modifies to a considerable degree the type of infection that develops on it. When urediospores were applied externally to the whole plant, the germ tubes did not come into contact with the most rapidly growing tissues of the plant and the whole plant ordinarily remained resistant, as Khapli to forms 21 and 36, Kota to forms 2 and 38,

TABLE II

TYPES OF INFECTION PRODUCED BY PHYSIOLOGIC FORMS OF *Puccinia graminis tritici* ON LEAVES AND SHEATHS OF WHEAT VARIETIES, (1) WHEN SPORES ARE APPLIED TO THE SURFACE OF MATURE TISSUE AND (2) WHEN SPORES ARE DEPOSITED IN IMMEDIATE CONTACT WITH RAPIDLY GROWING TISSUE

Variety	Number of plants tested	Form used	Spores applied to the surface of mature tissue		Spores deposited in immediate contact with rapidly growing tissue			
					Flag leaf		Flag-leaf sheath	
			Leaves	Sheaths	Basal portion	Apical portion	Lower portion	Upper portion
Immune								
Marquis × Kanred	10	21	(0)	(0)	(0) (;)	(0)	(0) (;)	(0)
Iumillo	10	36	(0)	(0)	(0) (;)	(0)	(0) (;)	(0)
Resistant								
Khapli	10	36	(1) −	(1) −	(3) (4)	(;) (1) −	(3) (4)	(;) (1) −
Khapli	16	21	(1) =	(1) =	(3) (4)	(;) (1) −	(3) (4)	(;) (1) −
Kota	10	2	(2) =	(2) =	(3) (4)	(;) (2)	(3) (4)	(;) (2) −
Kota	10	38	(1) +	(1) +	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Marquis	30	38	(2) =	(2) =	(3) (4)	(;) (1)	(3) (4)	(;) (2) −
Speltz Marz	18	36	(1) =	(1) =	(3) (4)	(;) (1)	(4)	(;) (1)
Indeterminate (mesothetic)								
Marquis	16	120	(x)	(x)	(3) (4)	(;) (x) −	(3) (4)	(;) (x) −
Speltz Marz	14	38	(x) +	(x) +	(3) (4)	(;) (2) −	(4)	(;) (1)

Marquis to form 38, and Speltz Marz to form 36. Similarly, when urediospores were applied externally to a semi-resistant plant (one which develops an "x" type of infection), as Marquis to form 120 and Speltz Marz to form 38, the whole plant remained fairly resistant at low or medium temperatures. When, however, the urediospores were injected by means of a hypodermic syringe into the tissues of the different internodes of plants in groups (ii) and (iii) mentioned above, the germ tubes came into contact with very rapidly growing tissue in the upper part of the plants, and some ten days later pustules of the "4" type developed on the sheaths of the upper part of these plants (Fig. 1, A); the sheaths of the lower part, however, were growing very slowly and remained resistant. That is to say, the tissues that were growing and elongating very rapidly were susceptible and large pustules developed upon them, whereas the tissues that were growing very slowly or had ceased to elongate were resistant and remained relatively free from infection.

As already indicated, each node at the time of inoculation by injection was enclosed by a sheath, but, by the time the pustules began to break out on the sheaths of the upper part of the plant, each node had pushed beyond the sheath. The heaviest infections occurred in the flag-leaf sheaths, those on the other sheaths being lighter. If the flag leaf was enclosed at the time of inoculation, a heavy infection developed on its basal portion.

The rust development on any sheath was not uniform with respect to pustule type. From the node upward to the base of the leaf blade there was

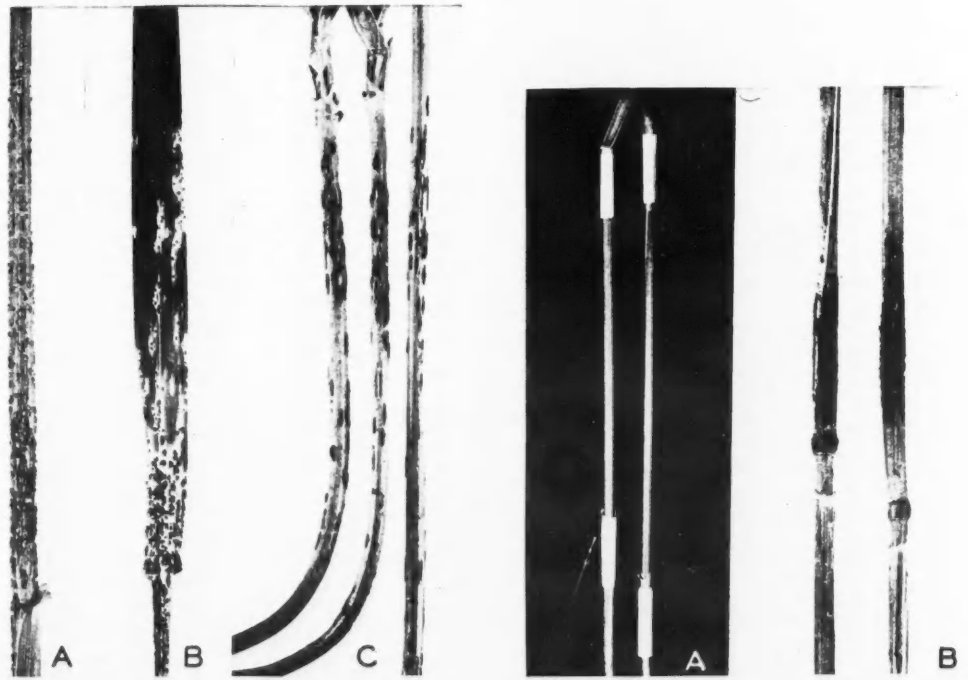


FIG. 1, A. *Types of infection on the sheath of Marquis wheat following inoculation by injection with P. graminis tritici form 38, a form to which Marquis is resistant.* FIG. 1, B. *Types of infection on the flag leaf of Peatland barley following inoculation by injection with P. graminis tritici form 21. Note in A and B the gradual transition from the susceptible type of pustule on the younger tissue at the base to the resistant type of pustule on the older tissue above.* FIG. 1, C. *Type of infection on the peduncle of Acme wheat. The peduncle was inoculated with form 21 while it was still very young and enclosed in the sheath. Similar peduncles after they had emerged from the sheath were quite resistant to this form.* FIG. 2, A. *The lower portion of two young culms of Acme wheat inoculated by placing dry spores inside the outer sheath. The white bands are pieces of gummed paper which keep the sheaths in their clasping position.* FIG. 2, B. *Infection on inner sheath above node on Acme wheat resulting from the inoculation mentioned in A. The outer sheath has been cut off a short distance below the node.*

PLATE I

a more or less gradual transition from the susceptible to the resistant type. The susceptible region varied in extent in different plants according to the succulence of the plant. In general, the more rapidly the plant was growing, the larger was the susceptible region; but even in the most rapidly growing plants, the susceptible region did not extend halfway up any internode and, usually, it involved only one or two inches of the sheath. In this region the pustules were of a susceptible or "4" type; while, directly above this region, the pustules became progressively smaller until they were of the resistant or "1" type, such as developed on the seedlings of the varieties in question. The presence of the "4" type of pustule upon the younger tissue of the upper part of the plants, and of the "1" type upon the older tissue seemed to suggest a correlation between the age of the tissue and the ease with which the organism could develop in it.

Although the correlation appeared to hold with all resistant and semi-resistant varieties, it did not seem to hold with the immune strain Marquis × Kanred inoculated with form 21, or Iumillo inoculated with form 36. Such varieties remained resistant despite the age of the tissue inoculated or the method of inoculation. The variety Iumillo was not genetically pure for rust resistance, and, as a consequence, a very small number of plants showing susceptibility were found in some of the trials.

As has already been stated, all the experiments were carried out at both a high (75° F.) and a low temperature (60° F.), for it was known that differences in temperature may bring about striking differences in type of rust infection. Waterhouse (26) working with *P. graminis tritici* called attention to the fact that environmental changes, especially temperature changes, may bring about fluctuations not only in the amount of stem rust development but also in the type of infection produced. Johnson (15) found that any physiologic form of stem rust which produces an "x" type of infection on durum wheats at ordinary greenhouse temperature was likely to produce on the same variety a "4" type of infection at high and a "1" to "0" type at low temperatures. Although, with physiologic forms which produced an "x" type of infection, the response to temperature was more pronounced than with other forms, nevertheless a large number of forms showed at least a slight response to temperature. It seemed quite possible, therefore, that at the two different temperatures (75° F. and 60° F.) the pustule size of the susceptible and resistant portions of the sheaths might not remain constant, especially as two physiologic forms were being used in the tests which produced an "x" type of infection on certain hosts, namely, form 38 on Speltz Marz and form 120 on Marquis. In order, therefore, to obviate any uncertainty in the interpretation of the results of the present experiments the plants were kept at the two temperatures specified.

An examination of the plants maintained at both temperatures disclosed no visible difference in the amount of rust development or in the type of pustule on the susceptible portions of the plants, or any difference in these

respects on the resistant portions. At both temperatures, the susceptible portions were almost covered with pustules of the "4" type, while the resistant portions had scattered pustules of "1" and "x" types.

Oat Varieties

As the experiments had shown that plants of the wheat varieties enumerated in Table II were resistant to certain physiologic forms of *P. graminis tritici* in the seedling stage when spores were applied to their exterior, but susceptible in the adult stage when spores of these same forms were injected into certain regions of the plants, an experiment was begun to discover if resistant varieties of oats might likewise exhibit regional susceptibility when spores of *P. graminis avenae* Erikss. and Henn., were injected into them.

For this experiment the oat varieties Green Russian, Joanette, and Richland were inoculated by injection with *P. graminis avenae* form 1; Belar, Red Rustproof, Ruakura, Sunrise, and Sterilis Selection, with *P. coronata avenae* form 3; and Anthony and White Russian, with *P. coronata avenae* form 4. All these varieties were known to be highly resistant in the seedling stage to the respective forms just enumerated. As will be seen from Table III, heavy infections developed on the culm above the nodes of the upper part of the plant and also on the basal portions of the upper leaves. As in the case of wheat, there was a tendency also for the pustules on the oat varieties to become progressively smaller from the node to the base of the leaf above, and from the base of the leaf to the tip. On an oat plant, however, the susceptible region was somewhat larger than on wheat, a condition probably attributable to the fact that the oat plants were in a more succulent state than were the wheat plants.

The oat experiment was also carried out both at a high (75° F.) and a low temperature (60° F.), in order to ascertain if two different temperatures would bring about any difference in the amount of rust development or in the type of pustule on the susceptible portions of the plants or any difference in these respects on the resistant portions. Peturson (21) had observed striking differences at these two temperatures in infections on seedlings inoculated with forms of *P. coronata avenae* that produce an "x" type of infection, and Gordon (9) had demonstrated a similar behavior for *P. graminis avenae* when forms that produce an "x" type of infection were used. With oats, as with wheat, no discernible differences were observed between the reactions of the susceptible areas, or of the resistant areas, at either temperature.

Barley Variety

As the experiments had shown that both wheat and oat plants in the seedling stage were resistant to certain physiologic forms of stem rust when spores were applied to their exterior, but susceptible when spores of these same forms were injected into them, it seemed quite probable that resistant barley varieties might likewise exhibit a regional susceptibility when spores of *P. graminis tritici* were injected into them.

TABLE III

TYPES OF INFECTION PRODUCED BY PHYSIOLOGIC FORMS OF *Puccinia graminis avenae* AND *P. coronata avenae* ON LEAVES AND SHEATHS OF OAT VARIETIES, (1) WHEN SPORES ARE APPLIED TO THE SURFACE OF MATURE TISSUE AND (2) WHEN SPORES ARE DEPOSITED IN IMMEDIATE CONTACT WITH RAPIDLY GROWING TISSUE

Variety	Number of plants tested	Rust	Form	Spores applied to the surface of mature tissue		Spores deposited in immediate contact with rapidly growing tissue			
						Flag leaf		Flag-leaf sheath	
				Leaves	Sheaths	Lower portion	Upper portion	Lower portion	Upper portion
Resistant									
Green Russian	6	*P. graminis avenae*	1	(1) (2) −	(1) (2) −	(3) (4)	(;) (2)	(3) (4)	(;) (2)
Richland	6	*P. graminis avenae*	1	(1) (2)	(1) (2)	(3) (4)	(;) (2)	(3) (4)	(;) (2)
Indeterminate									
Joanette	6	*P. graminis avenae*	1	(x) −	(x) −	(3) (4)	(x)	(3) (4)	(x)
Resistant									
Red Rustproof	4	*P. coronata avenae*	3	(;) (1) −	(;) (1) −	(3) (4)	(;) (1) −	(3) (4)	(;) (1) −
Ruakura	4	*P. coronata avenae*	3	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Sunrise	4	*P. coronata avenae*	3	(;) (1) −	(;) (1) −	(3) (4)	(;) (1) −	(3) (4)	(;) (1) −
Sterilis Selection	4	*P. coronata avenae*	3	(;) (1) −	(;) (1) −	(3) (4)	(;) (1) −	(3) (4)	(;) (1) −
Indeterminate (mesothetic)									
Anthony	4	*P. coronata avenae*	4	(x)	(x)	(3) (4)	(x) +	(3) (4)	(x) +
Belar	4	*P. coronata avenae*	3	(x)	(x)	(3) (4)	(x) +	(3) (4)	(x) +
White Russian	4	*P. coronata avenae*	4	(x)	(x)	(3) (4)	(x) +	(3) (4)	(x) +

Only one barley variety, Peatland, was known to be highly resistant to stem rust in the field. It was selected, therefore, for the test. Ten plants were inoculated by the injection method with *P. graminis tritici* form 21 and ten with form 36.

Results similar to those reported for wheat and oats were obtained. The lower part of each plant was resistant but some portions of the upper part were susceptible, especially that portion of the sheath that lies directly above the uppermost node. Numerous pustules of the susceptible type developed on it. There was a tendency also for the pustules to become progressively smaller from the node upwards to the base of the leaf, and from the base of the leaf to the tip (Fig. 1, B).

Regional Susceptibility in Adult Plants of Wheat Varieties Possessing Mature Plant Resistance

Some varieties of wheat in their early stages of growth are susceptible to certain physiologic forms of stem rust, but in later stages become highly resistant. For instance, Melchers and Parker (17) described a strain of Crimean Kansas which gave no evidence of resistance in the greenhouse but was partially resistant in the adult stage in the field. Hursh (14) called attention to the difference between the rust reactions of Acme seedlings and the reactions of that variety when approaching maturity in the field, and attributed these differences to greater amounts of sclerenchyma in the mature plants, which acted as a mechanical barrier to the growth of the rust mycelium. Goulden, Newton and Brown (11) studied the reactions of wheat varieties at two stages of maturity to sixteen physiologic forms of stem rust and, on the basis of their results, were able to divide these varieties into three groups: (1) varieties showing no evidence of mature plant resistance—Garnet, Marquis, Quality, and Khapli; (2) varieties showing varying degrees of mature plant resistance, from a mere indication to a very pronounced evidence—Reward, Kota, Black Persian, Hope, H-44-24, Pentad, and Acme; and (3) varieties showing no appreciable difference between the seedling and mature plant reactions but suspected of possessing considerable mature plant resistance owing to the very low percentage of rust which develops on them in the mature stage—Vernal emmer and Iumillo.

Goulden, Neatby and Welsh (10) in an inheritance study, pointed out that the mature plant resistance in H-44-24 × Marquis crosses and in a Pentad × Marquis cross was inherited independently of the physiological resistance observed in the seedling stage.

As there appeared to be a sharp distinction between the mature plant and the seedling type of resistance, the question naturally arose, would the young, rapidly elongating tissue in adult plants possessing mature plant resistance become infected if spores were deposited in immediate contact with it, as did such tissue in resistant plants possessing seedling resistance?

Adult plants of Hope and H-44-24, varieties known to be highly resistant, and of Pentad and Acme, varieties known to be quite resistant in the mature

stage to stem rust, were inoculated with a suspension of urediospores of form 21 in the manner already described. After the inoculated plants had remained two weeks in the warm house, or three weeks in the cold one, their reaction was very similar to that of the varieties resistant in the seedling stage. The remaining portions of the plants were quite resistant.

The experiment was repeated, but another form, namely, form 36, was used as inoculum. Very similar infection areas to those reported for form 21 developed on all the hosts except Pentad. For some unknown reason Pentad was more lightly infected, and in each test the proportion of plants which became infected was smaller than when form 21 was used.

TABLE IV

TYPES OF INFECTION PRODUCED ON THE LEAVES AND SHEATHS OF FOUR WHEAT VARIETIES POSSESSING MATURE PLANT RESISTANCE WHEN SPORES OF TWO PHYSIOLOGIC FORMS OF *Puccinia graminis tritici* ARE (1) APPLIED TO THE SURFACE OF MATURE TISSUE AND (2) DEPOSITED IN IMMEDIATE CONTACT WITH RAPIDLY GROWING TISSUE

Variety	Number of plants tested	Form used	Spores applied to the surface of mature tissue		Spores deposited in immediate contact with rapidly growing tissue			
					Flag leaf		Flag-leaf sheath	
			Leaves	Sheaths	Lower portion	Upper portion	Lower portion	Upper portion
Acme	30	21	(;) (1) +	(;) (1) +	(4)	(;) (2) +	(4)	(;) (2) +
Hope	40	21	(;) (1)	(;) (1)	(4) −	(;) (2)	(4) −	(;) (2)
H-44-24	40	21	(;) (1)	(;) (1)	(4) −	(;) (2)	(4) −	(;) (2)
Pentad	35	21	(;) (1)	(;) (1)	(4) −	(;) (2)	(4) −	(;) (2)
Acme	30	36	(;) (1) +	(;) (1) +	(4)	(;) (2)	(4)	(;) (2)
Hope	40	36	(;) (1)	(;) (1)	(3) +	(;) (1)	(3) +	(;) (1)
H-44-24	40	36	(;) (1)	(;) (1)	(3) +	(;) (1)	(3) +	(;) (1)
Pentad	60	36	(;) (1)	(;)	(3)	(;) (1)	(3)	(;) (1)

In most cases awns and glumes of plants of the varieties Hope, H-44-24, Acme, and Pentad were also heavily infected by both forms 36 and 21, but the necks were often free from infection. These findings were contrary to our expectations, for, while the necks were still enclosed in the sheaths, they grew extremely rapidly and should have contained a large amount of young tissue. Either the neck tissues were not susceptible or the inoculum did not come into contact with them. In order to determine this point, the sheaths were carefully stripped away from the necks of a number of plants and the necks inoculated in the ordinary way by applying spores to the surface. After the sheaths had been removed, it was found that the necks were too weak to support the heads and required mechanical support. The necks eventually became heavily infected (Fig. 1, C). It seems fairly safe to assume, therefore, that the absence of infection on the necks, already referred to, cannot be attributed to resistance in the neck tissues but rather to the failure of the inoculum to reach those tissues.

In further experiments it was found that, with increasing age, the necks became less and less susceptible and by the time they had fully emerged from the sheath they were highly resistant. The period of extreme susceptibility was thus comparatively short. It varied, however, to some extent with the condition of the plant. When the plant was very succulent and was growing extremely rapidly, the tissues of the neck remained susceptible for a longer period than when the plant was growing more slowly. In other words, regional susceptibility was always associated with young, rapidly growing tissue.

Infections on the Culm above the Node in Field and Greenhouse

Most investigators who have studied the rust reactions of wheat varieties in the field have called attention to the fact that pustules often appear on the culm just above the nodes before any pustules can be found on other parts of the plant.

Hart (13) made a careful morphological study of the sheath above the node as well as of other portions of the sheath and came to the conclusion that the large pustules above the nodes were due to the fact that the sclerenchymatous girders (stereomes) did not extend to the outer epidermis in this part as they did in other parts of the sheath, and, consequently, the mycelium of the rust could spread tangentially as well as lengthwise in the tissue and thus form larger pustules than were possible in the remainder of the sheath.

Her findings might account for the presence of large pustules just above the nodes of susceptible plants, but they did not seem to explain why, in most varieties exposed to natural infection, large pustules often appeared also on the sheaths just below the leaf blade where, as she showed, the stereomes did extend to the outer epidermis; or why, under conditions of natural infection, heavy infections often developed just above the nodes of wheat varieties possessing mature plant resistance, such as Hope and Acme, whose resistance she attributed to the behavior of their stomata.

An explanation was suggested by results already mentioned in this paper: heavy infections were obtained by injecting a suspension of spores inside the leaf sheaths. Might not the large pustules just referred to have arisen from spores which had settled inside the sheath?

Generally, of course, infection is supposed to take place through the stomata of the outer epidermis of the plants; and, as a rule, the sheaths, especially the upper ones, tightly enfold the culm so that the possibility of spores getting inside a sheath may appear somewhat remote. However, under field conditions, winds sway the plants to and fro, causing them to bend. Sometimes the leaves of one plant get entangled with the leaves and culms of other plants. There would, therefore, be a tendency for the leaf sheaths to become loosened somewhat from the culms, or drawn away from them, at least for periods of short duration. In such an eventuality the spores might fall, or be washed down inside the sheaths by dew or rain drops.

It has already been mentioned in this paper that until a wheat plant is about to come into head the leaf sheath originating at a node (except the uppermost one) extends upwards about one inch or more beyond the node next above, so that the latter node and the lower portion of the sheath arising from it are enfolded for a considerable length of time by the upper portion of the first-mentioned sheath. If, therefore, spores succeeded in getting inside the upper part of this sheath, they would have an opportunity, after germination, to infect the upper portion of this sheath and the basal portion (enfolded by it) of the other one. Subsequently, as the plant grew, the two infected areas would be gradually drawn apart, so that the infections on the two areas would appear to have originated from two entirely different lots of spores.

In order to ascertain if heavy infections would develop on the upper portion of one sheath and on the basal portion of the sheath next above if dry urediospores were deposited inside the former, the sheaths of plants of Hope, H-44-24, Acme and Pentad were carefully drawn away sufficiently far from the culms to permit the depositing of urediospores inside them. Some plants of each variety were inoculated in this manner with form 21 and others with form 36. The sheaths were then replaced in their normal clasping position. The ones on the upper parts of the plants held that position without difficulty, but the ones on the lower parts of the plants had to be kept in place by mucilaged bands (Fig. 2, A). Owing to transpiration there was always some moisture inside the sheaths so that once the spores had been deposited they germinated readily.

As has been stated, the nodes were still enclosed in the sheaths at the time of the inoculations, but in less than ten days they emerged from the sheaths, for at this time very rapid growth is taking place in the nodal regions, particularly of the upper part of the plant. By the time the nodes had emerged, heavy infections (Fig. 2, B) were found to be developing just above the nodes of the upper part of the plant, the heaviest infections being just above the topmost node. These infections were similar to those which had appeared on the same wheat varieties when a distilled-water suspension of urediospores was injected into the tissues. In many cases a heavy infection also occurred on the upper part of the sheaths, the portion inside of which the spores had been deposited. Apparently in these cases infection had taken place on the inner side of the sheaths and the rust mycelium had spread through the sheath tissue to form pustules on the outer surface. It, therefore, seems safe to assume that many of the large pustules which, under field conditions, develop just above the nodes and on the upper portion of the leaf sheaths arise from infections produced by spores which succeed in getting inside the leaf sheaths.

Infection of Cereals by Rusts that are not Natural Parasites on them

It has long been known that the germ tubes of spores of different rusts can enter the stomata of plants that are not natural hosts for such rusts and reach

a certain stage of development in the substomatal cavity, but, as far as we are aware, it has not yet been reported that such incipient infections ever develop further.

In 1905, Gibson (8) inoculated a large number of unrelated plants on their outer surfaces with spores of *Puccinia chrysanthemi* Roze, as well as of other rusts, and found that the germ tubes enter the stomata of the different plants in the same way as they do in the natural hosts of these rusts. Substomatal vesicles are formed which give rise to from one to six hyphae. However, in the subsequent course of development the hyphae do not form haustoria and in about four days lose their capacity for growth. She concluded that whenever a germ tube of any rust fungus enters any plant other than a natural host a struggle goes on resulting in the death both of the host (locally) and of the parasite.

Stakman (23) and Stakman and Piemeisel (24) inoculated certain cereals and grasses on the outer surfaces with *Puccinia graminis* and found that some of these hosts could be attacked by certain rusts that are not, normally, parasitic on them. For example, rye and oats were attacked very slightly by *P. graminis tritici*, and rye and barley, slightly by *P. graminis avenae*, very minute pustules being formed in each case.

As the infections by *P. graminis avenae* on oats and rye reported by Stakman, and Stakman and Piemeisel were really quite similar to the type of infection that occurs on any cereal when inoculated on the surface with a physiologic form to which it is resistant, it seemed to us quite possible that heavy infections would develop on oat and rye plants, as on resistant wheat plants, if spores of *P. graminis tritici* were deposited in close contact with the rapidly growing tissues of such plants; and, similarly, that heavy infections would develop on rye and barley if thus inoculated with spores of *P. graminis avenae.*

A large number of such tests were carried out using the above-named hosts and rusts, as well as some additional hosts and rusts. The results of some of these tests are presented in Table V. From them it will be seen, for example, that the young tissues of the adult plants of oats and rye were heavily attacked by *P. graminis tritici* forms 9, 21 and 36; and barley by *P. graminis avenae* forms 6, 7 and 8, and *P. triticina* forms 20 and 35, but the tissues of wheat remained resistant to *P. graminis avenae* forms 6, 7 and 8, and those of barley resistant to *P. coronata avenae* forms 3 and 4. As far as the work has gone the results seem to show that if a plant in the seedling stage is immune when inoculated on the surface with a certain rust, infection being indicated only by flecking, injections of this rust into the young tissues of the plant will not result in a susceptible type of infection; but, if, on the other hand, minute pustules develop on a plant in the seedling stage after being inoculated on the surface with a certain rust, a "4" type of pustule will develop above the upper nodes of such a plant if the inoculum is deposited in close contact with the young tissues.

TABLE V

TYPES OF INFECTION PRODUCED BY CERTAIN RUSTS ON CEREALS THAT ARE NOT THEIR NATURAL HOSTS

Variety	Rust used	Forms	Spores applied to the surface of mature tissue		Spores deposited in immediate contact with rapidly growing tissue			
					Flag leaf		Flag-leaf sheath	
			Leaves	Sheaths	Lower portion	Upper portion	Lower portion	Upper portion
Wheat								
Little Club	*P. gr. avenae*	6, 7, 8	(;)	(;)	(;) (1)	(;)	(;) (;)	(;)
Marquis × Kanred	*P. gr. avenae*	6, 7, 8	(;)	(;)	(;) (1)	(;)	(;) (1)	(;)
Speltz Marz	*P. gr. avenae*	6, 7, 8	(;)	(;)	(;) (1)	(;)	(;) (1)	(;)
Chul	*P. anomala*	1, 2	(;)	(;)	(;)	(;)	(;)	(;)
Garnet	*P. anomala*	1, 2	(;)	(;)	(;)	(;)	(;)	(;)
Little Club	*P. anomala*	1, 2	(;)	(;)	(;)	(;)	(;)	(;)
Oats								
Green Mountain	*P. gr. tritici*	9, 21, 36	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Ruakura	*P. gr. tritici*	9, 21, 36	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Victory	*P. gr. tritici*	9, 21, 36	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Ruakura	*P. triticina*	20, 35	(;)	(;)	(;)	(;)	(;)	(;)
Victory	*P. triticina*	20, 35	(;)	(;)	(;) (1)	(;)	(;) (1)	(;)
Barley								
Manchuria	*P. gr. avenae*	6, 7, 8	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
O.A.C. 21	*P. gr. avenae*	6, 7, 8	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Success	*P. gr. avenae*	6, 7, 8	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Manchuria	*P. triticina*	6, 7, 8	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
O.A.C. 21	*P. triticina*	20, 35	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Success	*P. triticina*	20, 35	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Manchuria	*P. coronata avenae*	3, 4	(;)	(;)	(;) (1)	(;)	(;) (1)	(;)
O.A.C. 21	*P. coronata avenae*	3, 4	(;)	(;)	(;) (1)	(;)	(;) (1)	(;)
O.A.C. 21	*P. helianthi*		(;)	(;)	(;)	(;)	(;)	(;)
O.A.C. 21	*P. taraxaci*		(;)	(;)	(;)	(;)	(;)	(;)
Rye								
Rosen	*P. gr. tritici*	9, 21, 36	(;)	(;)	(2) (3)	(;)	(2) (3)	(;)
Swedish	*P. gr. tritici*	9, 21, 36	(;)	(;)	(2) (3)	(;)	(2) (3)	(;)
Colorless	*P. gr. tritici*	9, 21, 36	(;)	(;)	(2) (3)	(;)	(2) (3)	(;)

Discussion

Many varieties of wheat are known to be resistant to one or more physiologic forms of stem rust and to be susceptible to other forms. A few varieties are susceptible in the seedling stage to certain forms but highly resistant in the adult stage to all forms. On the other hand, it has been affirmed (5) that the susceptibility of a variety increases with increasing age of the plants. In the present investigation, it is not so much a matter of varietal resistance or susceptibility to particular forms of stem rust, or of the resistance or susceptibility of plants at different stages of development, but of some portions of the same plant being highly resistant and other portions highly susceptible at one and the same time.

The fact that the young, rapidly growing portions of rust-resistant varieties and strains of cereals are susceptible to stem rust has little or no significance

with respect to the ability of these varieties and strains to withstand the disease under field conditions. The susceptible portions of the plants are generally quite effectively protected by plant parts that are highly resistant. Although, as already mentioned, large pustules of stem rust may occasionally occur above some nodes of the resistant plants, the yield of the plants thus affected is not reduced in any measurable degree.

Whether or not the young tissues of an adult plant are susceptible to a physiologic form or even to a different rust can usually be ascertained in advance by observing the infection type on the seedling plant following inoculation with the particular physiologic form or rust. In general it was found that if a plant in the seedling stage is immune when inoculated on the surface with a certain physiologic form or rust, infection being indicated only by flecking, injection of this rust into the young tissues of the plant will not result in a susceptible type of infection; but, if, on the other hand, minute pustules develop on a plant in the seedling stage after being inoculated on the surface with a certain rust, a "4" type of pustule will develop on the very rapidly growing portion of such a plant if the inoculum is deposited in close contact with the young tissue.

That young tissues should be more susceptible than older tissues to attacks by rust is not surprising for it has been repeatedly shown, with plants other than cereals, that immature leaves and tissues are often more susceptible to certain diseases than older tissues. For instance, Salmon (22) found that the fully matured leaves of *Euonymus japonicus* L. did not become infected by mildew (an obligate parasite like the rusts) although the young leaves were quite susceptible. Ballard and Volck (1) observed that the young leaves and twigs of apple trees are particularly heavily infected with mildew. It is known also that young leaves of *Berberis vulgaris* L. are susceptible to *P. graminis* while the older ones are resistant. In these instances, however, the cuticle of the leaf is penetrated directly by the organism while in cereals the rust organism enters by way of the stomatal openings. The cases cited, therefore, may be in no way comparable with the present one. A mechanical mode of penetration suggests, of course, a mechanical basis of resistance, although this does not necessarily follow.

A somewhat parallel case to that of young susceptible tissues in cereals is reported by Fitzpatrick (4). He found that *Taphrina deformans* (Fcl.) Tul. will not spread in the peach leaf after the latter has reached a certain stage of maturity and he concluded that the older tissues develop a definite resistance to the organism.

The nature of resistance to stem rust is very imperfectly or not at all understood. The resistance of some wheat varieties has been attributed, in part at least, (*a*) to the late opening of the stomata (13); (*b*) to the high ratio of sclerenchymatous to collenchymatous tissue in the culms (14); (*c*) to the production of some toxic substance by the host tissue in the immediate vicinity of the infections (3, 19, 20); and (*d*) to a lack of suitable nutriment in the host tissue for the fungus (7, 16). It is not proposed to review here

the work of the various investigators who have concerned themselves with the problem of the nature of resistance to stem rust. The literature has been recently reviewed by Chester (2). Mention will only be made of two investigations which may appear to have some bearing on the present work.

Hart (13) found that the stomata of some varieties that possess mature plant resistance, Hope and Acme, open later in the morning than those of the susceptible varieties, Little Club and Marquis, and she formulated the theory that the resistance of Hope and Acme in the field is due principally to the behavior of their stomata. As has already been stated, when plants of Hope and Acme were inoculated by injection before they came into head, pustules developed upon the sheaths of the upper portion of the plants, particularly upon the flag-leaf sheath. It will be remembered that at the time of inoculation, when the inoculum was being forced between the sheaths, the flag-leaf sheath was covered by an outer sheath but, by the time the pustules appeared, the flag-leaf sheath had pushed beyond this outer sheath into full view. The pustules on the flag-leaf sheath were not all of uniform size. Upon the susceptible (young) tissue just above the node the pustules were of a "4" type, while, upon the resistant (older) tissue higher up on the same sheath, they were of a "1" type. As pustules occurred both on the susceptible and resistant portion of the sheath, it would seem that resistance in the sheath of Hope and Acme cannot be due to the failure of the germ tubes to enter the stomata of the sheath but rather to some characteristic of the cells or of the protoplasm.

It has been shown by Hursh (14) that the internal structure of the plant may affect the relative shape of the pustules. He found that, in the peduncle of Kota wheat, broad strands of sclerenchyma separate the narrow strands of collenchyma and that when the peduncles were attacked by rust, the pustules, hemmed in on two sides by sclerenchymatous strands and unable to grow at right angles to the peduncle, developed into long narrow pustules rather than large and broad pustules characteristic of susceptible wheats. In the resistant portion of the sheath and peduncle of the plants inoculated by injection there was nothing in the shape of the pustule to suggest mechanical interference. The pustules were of the normal "1" type which occurs on resistant seedlings and were so minute that the morphology of the leaf or peduncle would not appear to have influenced their shape.

Hursh (14) has also shown that there is a high ratio of sclerenchymatous to collenchymatous tissue in the culm of some resistant wheats. In the present investigation a study was not made of the morphology of the susceptible tissues in the resistant plants tested, but it was found that prior to the emergence of the heads, the peduncles of the plants were very flexible and quite unable to support the heads without mechanical support. At this stage the peduncles were very susceptible. As they became older, and more rigid, they became highly resistant. Similarly the young, rapidly elongating, basal portion of the sheath was always susceptible while the older portion was resistant.

Susceptibility in these young plant parts where sclerenchymatous tissue is apparently not well developed and resistance in the older parts where this tissue must be well developed would seem to indicate that mechanical resistance was operative. However, some of the results recorded in this paper throw considerable doubt on this conclusion. The young tissues of all wheat varieties are not susceptible to all physiologic forms of stem rust, although susceptible to many forms. For instance, the young tissues of the immune strain Marquis × Kanred remain resistant to form 21 despite the age of the tissue inoculated or the method of inoculation used. Furthermore, the young tissues of a cereal, such as oats or barley, may be susceptible to a rust which is not a natural parasite on it, but the same tissue may be highly resistant to another rust which is also not a natural parasite of that cereal. The young tissues of the oat variety Ruakura are heavily attacked by *P. graminis tritici* forms 9, 21 and 36 but they are highly resistant to *P. tritícina* forms 20 and 35. If, in these two instances, the resistance is due to mechanical obstruction, it is difficult to explain why the young tissues should retain their resistance only to certain physiologic forms and rusts and not to others, for such obstructions should be effective against any form or rust.

The present experiments have shown that the young tissues of resistant wheat and barley plants are (with the exceptions mentioned earlier) susceptible to physiologic forms of wheat stem rust, and young tissues of resistant oat plants to stem rust of wheat as well as of oats. Furthermore, the young tissues of barley plants are susceptible to stem rust of oats and leaf rust of wheat. When the young tissues grow older and are therefore more fully differentiated, they become highly resistant to the physiologic forms or rusts indicated. As already implied, this resistance does not appear to be morphologic in nature. It seems more probable that, with advancing age, the protoplasm of the cells undergoes some change which renders the tissues highly resistant.

References

1. Ballard, W. S. and Volck, W. H. Apple powdery mildew and its control in the Pajaro Valley. U.S. Dept. Agr. Bull. 120. 1914.
2. Chester, K. S. The problem of acquired physiological immunity in plants. Quart. Rev. Biol. 8 : 129-154, 275-324. 1933.
3. Dufrenoy, J. L'inégale susceptibilité des Épiceas vis-à-vis du *Chrysomyxa rhododendri*. Compt. rend. soc. biol. 109 : 352-353. 1932.
4. Fitzpatrick, Randal E. The life history and parasitism of *Taphrina deformans*. Sci. Agr. 14 : 305-326. 1934.
5. Gassner, G. Über Verschiebungen der Rostresistenz während der Entwicklung der Getreidepflanzen. Phytopath. Z. 4 : 549-596. 1932.
6. Gassner, G. and Hassebrauk, K. Über die Beeinflussung der Rostanfälligkeit durch Eintauchen geimpfter Blätter in Lösungen von Mineralsalzen und anderen Stoffen. Phytopath. Z. 5 : 323-342. 1933.
7. Gassner, G. and Franke, W. Der Stickstoffhaushalt junger Weizenpflanzen in seiner Abhängigkeit von der Mineralsalzernährung. Phytopath. Z. 7 : 187-222. 1934.
8. Gibson, C. M. Notes on infection experiments with various Uredineae. New Phytologist, 3 : 184-191. 1904.
9. Gordon, W. L. Effect of temperature on host reactions to physiologic forms of *Puccinia graminis avenae* Erikss. and Henn. Sci. Agr. 11 : 95-103. 1930.

10. GOULDEN, C. H., NEATBY, K. W. and WELSH, J. N. The inheritance of resistance to *Puccinia graminis tritici* in a cross between two varieties of *Triticum vulgare*. Phytopathology, 18 : 631-658. 1928.

11. GOULDEN, C. H., NEWTON, MARGARET, and BROWN, A. M. The reaction of wheat varieties at two stages of maturity to sixteen physiologic forms of *Puccinia graminis tritici*. Sci. Agr. 11 : 9-25. 1930.

12. HANNA, W. F. Studies on the nature of rust resistance in wheat. V. Physiology of the host. Can. J. Research, 4 : 134-147. 1931.

13. HART, HELEN. Morphologic and physiologic studies on stem-rust resistance in cereals. U.S. Dept. Agr. Tech. Bull. 266. 1931.

14. HURSH, C. R. Morphological and physiological studies on the resistance of wheat to *Puccinia graminis tritici* Erikss. and Henn. J. Agr. Research, 27 : 381-412. 1924.

15. JOHNSON, THORVALDUR. A study of the effect of environmental factors on the variability of physiologic forms of *Puccinia graminis tritici* Erikss. and Henn. Dominion of Canada Dept. of Agr. Bull. 140, New Series. 1931.

16. LEACH, J. G. The parasitism of *Puccinia graminis tritici* Erikss. and Henn. and *Puccinia graminis tritici-compacti* Stak. and Piem. Phytopathology, 9 : 59-88. 1919.

17. MELCHERS, L. E. and PARKER, J. H. Rust resistance in winter-wheat varieties. U.S. Dept. Agr. Bull. 1046. 1922.

18. NEWTON, MARGARET, and BROWN, A. M. Studies on the resistance of wheat and oats to stem rust. (Abst.) Phytopathology, 23 : 25. 1933.

19. NEWTON, R., LEHMANN, J. V. and CLARKE, A. E. Studies on the nature of rust resistance in wheat. I. General introduction. II. Physico-chemical properties of host-cell contents. III. Culture and injection experiments to demonstrate inhibiting or accessory substances. Can. J. Research, 1 : 5-35. 1929.

20. NEWTON, R. and ANDERSON, J. A. Studies on the nature of rust resistance in wheat. IV. Phenolic compounds of the wheat plant. Can. J. Research, 1 : 86-99. 1929.

21. PETURSON, B. Effect of temperature on host reactions to physiologic forms of *Puccinia coronata avenae*. Sci. Agr. 11 : 104-110. 1930.

22. SALMON, E. S. On a fungus disease of *Euonymus japonicus* L. J. Roy. Hort. Soc. 29 : 434-442. 1905.

23. STAKMAN, E. C. A study in cereal rusts. Physiological races. Minn. Agr. Exp. Sta. Bull. 138. 1914.

24. STAKMAN, E. C., and PIEMEISEL, F. J. Biologic forms of *Puccinia graminis* on cereals and grasses. J. Agr. Research, 10 : 429-495. 1917.

25. STAKMAN, E. C. and LEVINE, M. N. The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. Minn. Agr. Exp. Sta. Tech. Bull. 8. 1922.

26. WATERHOUSE, W. L. Australian rust studies. I. Proc. Linn. Soc. N.S.W. 54 : 615-680. 1929.

27. ZEHNER, MARION GRIFFITHS, and HUMPHREY, HARRY B. Smuts and rusts produced in cereals by hypodermic injection of inoculum. J. Agr. Research, 38 : 623-627. 1929.

STUDIES ON THE NATURE OF DISEASE RESISTANCE IN CEREALS

II. THE RELATIONSHIP BETWEEN SUGAR CONTENT AND REACTION TO STEM RUST OF MATURE AND IMMATURE TISSUES OF THE WHEAT PLANT[1]

By T. Johnson[2] and O. Johnson[3]

Abstract

In Part I of these studies it has been shown that the rapidly growing tissues of the wheat plant are more susceptible to stem rust than the older tissues. An attempt was made to discover if a physiological or chemical basis could be found for the difference in reaction of the young and older tissues.

Analyses were made to determine the sugar content of young (susceptible) and older (resistant) tissues of four wheat varieties resistant in the adult stage and of the corresponding plant parts of three wheat varieties which in the adult stage showed little or no resistance to rust. The young tissues comprised the young leaves still enfolded by the uppermost sheaths and the young stems below the uppermost node; the older tissues were represented by the fully developed upper leaves and their adherent sheaths. The analyses showed a considerably higher content of sugars in the young than in the older tissues of the seven varieties tested. The difference was particularly great in the content of reducing sugars but rather slight in the disaccharide content (expressed as invert sugar). However, as all the varieties, irrespective of resistance or susceptibility to rust in the adult stage, showed much the same difference in the sugar content of their young and older tissues, it does not seem likely that there is any direct relation between sugar content and reaction to rust.

Introduction

It has been demonstrated, in Part I of these studies (3), that the rapidly growing tissues of the wheat plant are decidedly more susceptible to stem rust than the older tissues. The work presented here originated as an attempt to discover if a physiological or chemical basis could be found for this radical difference in reaction.

The possibility that differences might exist between the concentration of sugar in the older, but photosynthetically active, parts of the plant and the younger, rapidly growing parts was first suggested by some experiments on the comparative osmotic values of these tissues. Sap was expressed, at a pressure of 6,000 lb. per sq. in., from fully developed leaves, and from young leaves still folded within the uppermost sheaths of wheat plants grown in the greenhouse. The osmotic pressure (determined by the vapor pressure method of Barger (1)) of the sap expressed from the young leaves proved to be considerably greater than that of the sap expressed from the fully mature leaves. The osmotic pressure of the sap expressed from fully developed leaves of the highly resistant varieties Acme, Iumillo, and H-44-24, was found equivalent to 6.7 atm. while the corresponding figure for the sap of the young leaves was 11.2 atm. The susceptible variety Little Club showed somewhat

[1] *Manuscript received July 27, 1934.*
Contribution No. 408 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.
[2] *Plant Pathologist, Dominion Rust Research Laboratory, Winnipeg, Canada.*
[3] *Plant Disease Investigator, Dominion Rust Research Laboratory, Winnipeg, Canada.*

similar, but slightly lower, osmotic values. The difference in the osmotic values of the two tissues, about 4.5 atm., was sufficiently great to indicate the possibility of some considerable difference in sugar concentration.

That carbohydrates play an important role in the development of rust is generally recognized. Waters (6), as a result of experiments on the urediospore and teliospore formation of ten species of rust, concluded that "all the rusts studied are directly dependent upon the photosynthetic activity of the host". Mains (2), however, has shown that *Puccinia Sorghi* Schw. and *Puccinia coronata* Cda. can develop in the absence of light when sugars are added to the nutrient solutions supplied to the host plants. It was, therefore, thought possible that a correlation might be found between the amounts of sugar present in the different parts of wheat plants and the reaction to stem rust of these plant parts. If, for example, a variety, such as Hope, were found to contain far greater quantities of sugar in its susceptible than in its resistant parts, while a variety, such as Little Club, which is more homogeneous in its reaction to rust, were found to be rather homogeneous in its sugar content also, one might be justified in drawing the conclusion that there was a positive correlation between the reaction of the various plant tissues to rust and their sugar content.

With a view of determining whether or not such a correlation existed, several wheat varieties which were thought to be suitable were selected and grown in plots adjacent to the laboratory to provide material for analyses of the sugar content of the various tissues. The selected varieties included four varieties possessing what is commonly described as mature plant resistance, namely, two varieties of *Triticum vulgare*, Hope and H-44-24, and two varieties of *Triticum durum*, Acme and Pentad. These wheats, as they approach the heading stage, are highly resistant to stem rust in their exposed plant parts but show susceptibility in the partly differentiated and young tissues protected by the outer sheaths. There were also included two varieties of *Triticum vulgare*, Marquis and Garnet, which do not possess mature plant resistance and are therefore susceptible to a great number, although not to all, of the physiologic forms of wheat stem rust. Finally, there was also included the variety Little Club (*Triticum compactum*) which is susceptible in all its stages of development and in all regions of the plant to the great majority of the known physiologic forms of *Puccinia graminis tritici*.

The homogeneity of the reaction of the various plant parts of Little Club, Marquis, and Garnet, holds, however, only for the physiologic forms to which they are susceptible. Experiments with forms to which their plant parts, when inoculated on the surface, are resistant, show that the rapidly growing regions protected by sheaths are only moderately resistant or even susceptible when inoculated by injection (3).

Methods

Plant material was collected for analysis in the last week of June, 1933, all collections being made at the same time of day, between 1:30 and 2:00 p.m.

Each of the seven varieties used was collected at approximately the same stage of development, that is, prior to the appearance of the flag leaf. At the time of collection the young head—in most cases less than one inch in length—was still enfolded within the lower part of the then uppermost sheath of the plant. There was therefore a considerable amount of rapidly growing tissue within this sheath and the one next below it, namely, the leaves folded within the uppermost sheath and the tender, young stem below the uppermost node.

The problem of separating the young, rapidly growing tissues from the fully developed tissues presented greater difficulties and greater opportunities for error than any other phase of the work. There is clearly no point of sharp demarcation between young and fully developed tissue and hence it is evident that any separation of such tissues must be more or less arbitrary. In the procedure adopted, the whole plant was not used for analysis, only such plant parts being used as were definitely known to be, respectively, mature and immature. As mature plant parts, the fully developed upper leaves of the plant together with their adherent sheaths were selected for analysis, while the immature plant parts comprised the young leaves still enfolded by the uppermost sheath and the young stem below the uppermost node.

The procedure actually used was briefly as follows. The plants were cut off near the base, placed upright in a large glass jar containing a quarter of an inch of water, and brought into a cool shady room where the tissues were separated out as quickly as possible. The leaves with their adherent sheaths were stripped off and dropped into a covered glass jar lined with moist blotting paper which maintained a humidity high enough to prevent a serious loss of water from the detached leaves. The young tissues, folded leaves and young stems, were separated out as rapidly as possible and dropped into a similarly prepared glass jar. When about 45 gm. of each of the two tissues had been collected (an operation requiring about 20 min. for two men), both lots were cut into short lengths with scissors, and 25 gm. of each was weighed out for analysis while three 5-gm. portions were used for dry-weight determinations. The 25-gm. lots to be used for analysis were immediately dropped into Erlenmeyer flasks containing 1 gm. of powdered calcium carbonate and 105 cc. of 95% ethyl alcohol. A reflux condenser was attached to the flask and the contents was boiled gently for 30 min. over a steam bath. Samples which could not immediately be subjected to extraction and analysis were stored in a refrigerator at about 5° C. until extraction could be carried out.

Extraction of sugars was performed in a continuous-extraction apparatus of the Soxhlet type, as modified by Newton (4). The sugars were extracted with 85% ethyl alcohol for a period of about 30 hr. The extract was transferred to a Claissen distilling flask, and concentrated under diminished pressure over a steam bath. About 200 cc. of distilled water was added when the contents of the distilling flask had been reduced to a volume of about 75 cc., and further concentration was carried out. The re-concentrated extract was then transferred to a 250 cc. volumetric flask, the concentrate being filtered through a pad of cheesecloth. Clarification was carried out by

adding 5 cc. of a saturated solution of neutral lead acetate, a quantity sufficient to leave in the solution a slight excess of lead acetate. The solution was then made up to volume with distilled water at 20° C. and filtered through a dry filter to remove the precipitate. The filtrate was deleaded by the use of a minimum quantity of powdered sodium oxalate which was added in small portions until precipitation was complete. The solution was then filtered through a dry filter and the filtrate used for the determination of reducing and total sugars. For the determination of total sugars, inversion was carried out by adding 5 cc. of concentrated hydrochloric acid to a 50 cc. aliquot of the neutralized filtrate which was then allowed to stand at room temperature (about 21–24° C.) for 24 hr. A single inversion was made for each extract and the determinations of reducing sugars before and after inversion were carried out in duplicate according to the Quisumbing and Thomas (5) procedure, employing direct weighing of the cuprous oxide.

TABLE I

SUGAR PRESENT IN YOUNG AND OLDER TISSUES OF WHEAT VARIETIES

—	Variety	Type of tissue	Green weight				Dry weight	
			Reducing sugars as dextrose, %	Ratio $\frac{\text{Young}}{\text{Old}}$	Sucrose as invert sugar, %	Ratio $\frac{\text{Young}}{\text{Old}}$	Reducing sugars as dextrose, %	Sucrose as invert sugar, %
Varieties developing mature plant resistance	Hope	Young	1.11	4.44	0.42	0.84	7.25	2.74
		Old	0.25		0.50		1.21	2.43
	H-44-24	Young	2.58	6.79	0.87	1.61	14.12	4.76
		Old	0.38		0.54		1.75	2.46
	Acme	Young	1.39	3.02	0.81	0.82	8.55	4.97
		Old	0.46		0.99		2.19	4.72
	Pentad	Young	2.25	2.74	1.24	1.17	12.29	6.77
		Old	0.82		1.06		3.95	5.10
Varieties not developing mature plant resistance	Marquis	Young	2.62	5.69	1.24	1.70	14.99	7.09
		Old	0.46		0.73		2.08	3.16
	Garnet	Young	2.40	4.61	1.19	1.65	13.86	6.87
		Old	0.52		0.72		2.27	3.11
	Little Club	Young	1.58	3.51	0.75	1.03	9.97	4.71
		Old	0.45		0.73		2.30	3.72

Discussion of Results

Before entering into a discussion of the possibility of a relationship between the sugar content of plant parts and their reaction to rust, attention should be called to the rather striking differences between the sugar contents of the varieties tested (Table I). The reason for these differences is not clear

although there are at least three possible causal factors. The differences may be due (i) to varietal characteristics, (ii) to the fact that the different varieties were collected for analysis on different days and under slightly different weather conditions, (iii) to slight differences in the age of the material collected. If the differences in sugar concentration were varietal characteristics, it would be expected that two varieties as closely related and similar in appearance as Hope and H-44-24 would also show a somewhat similar sugar content, and that the *vulgare* varieties Marquis and Garnet or the *durum* varieties Acme and Pentad would show some similarities in this respect. The data in Table I do not indicate any close resemblance in the sugar content of related varieties with the exception of the *vulgare* varieties Marquis and Garnet which give very similar results. There is therefore not much evidence that the differences are varietal characters. The second possibility, namely, that the environmental conditions at the time of collection of the plant material modified its sugar content to some extent cannot be overlooked. Although the plant material was in every case collected at the same time of day it is possible that the weather conditions from day to day varied sufficiently to affect to some degree the sugar content of the plants. Finally it is possible that slight differences in age of the plant parts at the time of collection had some influence on their sugar content. This would apply particularly to the younger or immature plant parts which, as already stated, had to be selected more or less arbitrarily. The varietal differences in sugar content are not, however, of any great significance in the present study, the object of which was not a comparison of the sugar content of the different varieties but rather a comparison of the sugar content of two different types of tissue in the same plant.

It is evident from an examination of Table I that the concentration of sugars is decidedly greater in the immature tissues than in the more mature tissues of the wheat varieties investigated. The differences in the amounts of reducing sugars present in the two types of tissues are particularly striking, these sugars being especially abundant, as might be expected, in the less mature regions of the plant, where rapid growth is taking place. To facilitate a comparison, the relationship between the sugar content of the young and older tissues of each variety has been expressed in the form of ratios. Thus in the variety Hope there is 4.44 times as much reducing sugar present in the young as in the older tissue. In view of these differences it would appear that there is some relation between the reaction to rust and the sugar content of the tissues in question, as the plant parts high in sugar content are also susceptible to rust. But all the varieties studied, irrespective of whether they do or do not possess mature plant resistance, show much the same differences in the sugar content of the two types of tissues. Further, it will be observed that, in the varieties Marquis, Garnet, and Little Club, the older plant parts, which are also susceptible to rust, are comparatively low in sugar content; in fact, their sugar content is no higher than that of the corresponding, but highly resistant, plant parts of Acme and Pentad. These observa-

tions throw considerable doubt on the existence of any relation between susceptibility to rust and sugar content, a doubt further confirmed by a consideration of the ratios in column 5 of Table I.

It is obvious from an examination of these ratios that the varieties do not fall into groups according to their rust reaction. In the comparison of the reducing-sugar contents of the two types of tissues, the highest ratio, 6.79, is found in the highly resistant variety H-44-24 which is, however, followed by the susceptible varieties, Marquis with a ratio of 5.69, and Garnet with 4.61. The lowest ratios are given by the resistant varieties Pentad, 2.74, and Acme, 3.02. Similarly, an examination of the ratios for the comparative contents of sucrose fails to show any grouping of these varieties according to reaction to rust. It does not appear, therefore, from this point of view, that there is any evidence that the differences in the sugar content of young and older regions of the plant have any direct relation to the reaction of these regions to stem rust.

Although the above conclusion seems valid for most of the physiologic forms of the stem-rust organism, it does not seem to hold for all the known physiologic forms. Certain forms, to which the leaves of Marquis, Garnet and Little Club are resistant, are capable of attacking, to a certain extent at least, the younger plant parts enclosed within the sheaths. As these younger regions are higher in sugar content than the leaves it follows that, in these instances, there is a correlation between sugar content and reaction to rust. It should be pointed out, however, that there is no proof that the higher sugar content of the younger plant parts is the cause of their greater susceptibility.

Apart from any consideration of its relationship to rust reaction, the preponderance of sugars in the rapidly growing tissues of the wheat plant is of some interest. It accords with the expectations based on the osmotic pressure determinations previously mentioned and is one of the factors contributing to the higher osmotic values of the sap extracted from the younger tissues. As the osmotic values of the extract were considerably lower for the older tissues (photosynthetically active leaves) than for the younger tissues, it follows that if dissolved materials are moving from the leaves to the rapidly growing plant parts they must be moving against an osmotic gradient. The results reported in the present paper indicate not only translocation of sugars against an osmotic gradient but also against a concentration gradient of sugars.

In addition to the differences in total sugar content of the young and older plant parts there is also a considerable difference in the quantitative relationships of disaccharides and monosaccharides in the two types of tissue. In the older tissues the proportion of sugars present as disaccharides is without exception greater than in the younger, namely, from 25.22 to 36.82% in the young tissues, and from 56.38 to 68.27% in the older tissues of the seven varieties tested.

Acknowledgments

The writers wish to express their gratitude to Dr. W. F. Geddes for placing at their disposal the facilities of his laboratory and equipment in the Chemistry Department of the University of Manitoba. They also wish to thank Mr. D. S. Binnington for assistance in the construction of the modified Soxhlet extraction apparatus.

References

1. BARGER, G. A microscopical method of determining molecular weights. J. Chem. Soc. London, 85 : 286-324. 1904.
2. MAINS, E. B. The relation of some rusts to the physiology of their hosts. Am. J. Botany, 4 : 179-220. 1917.
3. NEWTON, MARGARET, and BROWN, A. M. Studies on the nature of disease resistance in cereals. I. The reactions to rust of mature and immature tissues. Can. J. Research, 11 : 564-581. 1934.
4. NEWTON, R. A comparative study of winter wheat varieties with special reference to winter-killing. J. Agr. Sci. 12 : 1-19. 1922.
5. QUISUMBING, P. A. and THOMAS, A. W. Conditions affecting the quantitative determination of reducing sugars by Fehling's solution. J. Am. Chem. Soc. 43 : 1503-1526. 1921.
6. WATERS, CHARLES W. The control of teliospore and urediniospore formation by experimental methods. Phytopathology, 18 : 157-213. 1928.

A SIMPLE METHOD FOR DETERMINING THE RELATIVE WEIGHT PER BUSHEL OF THE GRAIN FROM INDIVIDUAL WHEAT PLANTS[1]

By O. S. Aamodt[2] and J. H. Torrie[3]

Abstract

The kernels from individual plants in studies on breeding for drought resistance varied so greatly in size that the usual measure of plumpness (weight per thousand kernels) was of little value. A method for determining the weight per measured bushel of small samples was developed and found to have a very high degree of association with an estimated degree of kernel plumpness. The weight in grams of a 4 cc. sample multiplied by 20, gave a close approximation to the test weight per bushel as determined by the usual apparatus. The correlation coefficient calculated from the weight per bushel of 184 samples of spring wheat by these two methods was $+0.947 \pm 0.005$ and for 59 samples of winter wheat $+0.834 \pm 0.027$.

In the course of drought breeding studies carried on at the University of Alberta, it was found advisable to devise some simple method for determining the relative weight per bushel of the grain from individual wheat plants. The weight per bushel gives an excellent measure of the plumpness of grain for any given sample of wheat. In studying plumpness with large samples several indicators are commonly used, namely, test weight per bushel, weight per thousand kernels and the percentage of plump kernels.

Weight per bushel as an index of flour yielding capacity of wheat was used by the practical miller before the advent of the present grading system. It is now regarded as an important factor in determining the yield of flour to be expected from a sample of wheat. The grading systems in use in both Canada and the United States give the test weight per bushel an important place in determining the grade of the wheat. Kernel plumpness, a desirable feature of wheat from the miller's point of view, is difficult to measure accurately, but, as shown later in this paper, is correlated with weight per bushel. Weight per thousand kernels is not, as far as the writers know, closely related to the flour yielding capacity or general milling quality of a wheat.

Bridgford and Hayes (1) with rod-row trials of spring wheat found a correlation of $+0.36 \pm 0.08$ between per cent plumpness and weight per thousand kernels. Clark and Smith (2) studying the F_3 of crosses between Pentad and Akrona report the following correlations: between test weight per bushel and plumpness percentage, $+0.627 \pm 0.048$; between test weight per bushel and weight per thousand kernels, $+0.468 \pm 0.062$; and between weight per thousand kernels and plumpness percentage, $+0.635 \pm 0.034$. Waldron (3) with rod-row trials, under conditions of high temperature and low moisture,

[1] *Manuscript received July 10, 1934.*
Contribution from the Department of Field Crops, University of Alberta, Edmonton, Canada, with financial assistance from the National Research Council of Canada. Published as paper No. 64 of the Associate Committee on Grain Research.
[2] *Professor of Genetics and Plant Breeding, University of Alberta.*
[3] *Graduate Assistant, Department of Field Crops, University of Alberta.*

obtained the following correlations: between test weight per bushel and plumpness percentage, +0.664; between test weight per bushel and weight per thousand kernels, +0.341; and between weight per thousand kernels and plumpness percentage, +0.630.

The parents used in the drought studies differed considerably in shape and size of the kernel. Milturum .0321 has a long, narrow kernel, while that of Selection I-28-60 is relatively short and broad. The hybrids showed considerable variation in kernel shape and size. Some samples with large, badly shrivelled kernels had the same weight per thousand kernels as other samples with small plump kernels. Weight per thousand kernels does not differentiate between the two characters of kernel plumpness and size. It is also a laborious method involving considerable time and expense. For these reasons the weight per thousand kernels was not considered to be a satisfactory measure of kernel plumpness.

Thus the necessity arose of devising some simple method which would measure these two characters for a sample of grain from a single plant, and at the same time overcome the discrepancy introduced into the determination of plumpness by differences in kernel size. Since, commercially, wheats are evaluated to a considerable extent by their weight per measured bushel, it seemed desirable to determine the weight of these small samples in units that could readily be converted by a simple conversion factor into weight in pounds per measured bushel.

Many of the grain samples from individual plants grown in the drought area had a volume of only a few cubic centimetres. Consequently, the maximum size of the measure to be used for obtaining the relative weight of the grain would be determined by the amount of grain produced by the plants most severely affected by the drought. Those plants that had the kernels developed sufficiently well to be considered as having produced a crop of grain, would have a minimum volume of approximately 4–5 cc. Since there are 36,369 cc. in a bushel, and 453.6 grams in a pound, the weight in grams of 80.2 cc. is the same as the weight in pounds of one bushel. One-twentieth of this volume, or 4.01 cc., would be a suitable volume for measuring the grain from individual plants, and at the same time provide a simple conversion factor by which the weight of 4.01 cc. of grain, in grams, could be expressed directly in pounds per bushel.

A 25 cc. graduate, cut off at the 4 cc. point, was used to measure the grain from individual plants. The weight of the 4 cc. of grain in grams, times twenty, gave a good approximation of the weight per bushel in pounds. The grain was poured into the measure from a coin envelope, pressed lightly with the thumb, and then levelled off with a scalpel drawn across the top of the measure.

In order to compare the accuracy of the 4 cc. measure with the pint weight-per-bushel measure, weights per measured bushel were determined by both methods for 184 samples, including 69 varieties, of spring wheat; and 59 samples, including 46 varieties, of winter wheat. All of these samples were

taken from the regular varietal plots at Edmonton. One determination was made with the pint measure and four with the 4 cc. measure. In the latter case the average of the four determinations was used as the value for the weight of the sample. The probable error for the quadruplicated 4 cc. determinations for spring wheat was $\pm 0.28\%$, and for the winter wheat samples it was $\pm 0.34\%$.

The range of the spring wheats in pounds per measured bushel for the 4 cc. measure was from 60.5 to 68.5 lb., while the range for the same samples in the pint measure was from 59.0 to 67.0 lb. The correlation coefficient calculated from the weights obtained on the 184 samples of spring wheat by these two methods was $+0.947 \pm 0.005$ (Table I). The range of the

TABLE I

CORRELATION TABLE FOR 184 SAMPLES OF SPRING WHEAT, SHOWING THE ASSOCIATION BETWEEN THE TEST WEIGHT PER BUSHEL, OBTAINED BY THE PINT WEIGHT PER BUSHEL MEASURE AND THE 4 CC. GRADUATE MEASURE

Test weight per bushel by the pint measure (x)

Test weight per bushel by the 4 cc. graduate measure (y)	59.0	59.5	60.0	60.5	61.0	61.5	62.0	62.5	63.0	63.5	64.0	64.5	65.0	65.5	66.0	66.5	67.0	f
60.5	1																	1
61.0		1																1
61.5	1																	1
62.0																		0
62.5			5		1													6
63.0							2	1										3
63.5					1		1											2
64.0							2	3	1									6
64.5						1	5	3	6	3	1		1					20
65.0								2	5	4	8		3					22
65.5								2	4	9	7	7	1					30
66.0									2	4	7	5	5	3				26
66.5										1	5	6	9		2			23
67.0											1	6	6	5	3	1		22
67.5												2	6	2	4		1	15
68.0															2			2
68.5															2		2	4
fx	2	1	5	0	2	1	10	11	18	21	29	26	31	10	13	1	3	184

$r = 0.947 \pm 0.005$

winter wheats in pounds per measured bushel for the 4 cc. measure was from 62.5 to 68.0 lb., while the range for the same samples in the pint measure was from 60.5 to 67.0 lb. The correlation coefficient calculated from the weights obtained on the same 59 samples of winter wheat by these two methods was $+0.834 \pm 0.027$.

In both of the above comparisons, it will be noted that the weights obtained with the 4 cc. measure are slightly higher than those obtained with the pint measure. The differences are so small that when placed on a percentage basis they are for all practical purposes really insignificant.

For the spring and winter wheat samples the regression equations of weight per bushel by the pint apparatus on weight per bushel by the 4 cc. method are: $x=0.933y+2.23$ and $x=0.939y+2.56$ respectively, where x = weight per bushel by the pint apparatus and y = weight per bushel by the 4 cc. method. The standard errors of prediction of the pint result, using the 4 cc. results are: 0.04 lb. for the spring wheat samples and 0.12 lb. for the winter wheat samples. These equations show the relationship existing between the two methods for obtaining the weight per bushel, thus enabling the result of one method to be expressed in terms of the other.

The high degree of correlation in the relative weights per measured bushel of different wheat samples, obtained by the pint and 4 cc. measures indicates that the latter measure is sufficiently reliable to be a useful instrument in determining the weight per bushel of small samples.

Kernel plumpness was determined by three methods for 315 F_3, lines, from a cross between Selection I-28-60 and Milturum, grown on dry land at Brooks, Alberta, in 1932. The methods used were: (i) weight per thousand kernels, (ii) weight per 4 cc. converted to weight per measured bushel, and (iii) an estimated kernel plumpness. The latter was determined by estimating the average degree of plumpness of the kernels in each sample, and assigning it a value of from 1 to 10, 1 representing completely shrivelled, and 10 completely plump. Intermediate degrees of plumpness were assigned values lying between 1 and 10. Correlation coefficients between each pair of the three methods for determining kernel plumpness were calculated from the data obtained. These are given in Table II.

TABLE II

CORRELATION BETWEEN DIFFERENT METHODS OF DETERMINING KERNEL PLUMPNESS IN 315 F_3 LINES, FROM A CROSS BETWEEN SELECTION I-28-60 AND MILTURUM

Variables correlated	r
Weight per thousand kernels and weight per bushel (4 cc.)	$+0.360 \pm 0.033$
Weight per thousand kernels and kernel plumpness	$+0.478 \pm 0.034$
Weight per bushel (4 cc.) and kernel plumpness	$+0.872 \pm 0.011$

There is a fair degree of correlation indicated when weight per thousand kernels is correlated with weight per bushel, $+0.360 \pm 0.033$; and with kernel plumpness $+0.478 \pm 0.034$. These results are, in general, in agreement with those reported by Bridgford and Hayes (1), Clark and Smith (2) and Waldron (3). A high degree of correlation was obtained when weight per bushel, as obtained with the 4 cc. graduate measure, was correlated with an estimated kernel plumpness, $+0.872 \pm 0.011$. These two methods appear to be the most reliable for determining the degree of shrivelling of wheat kernels in small samples.

Conclusions

When the plumpness of a large number of samples is to be determined, and an experienced person is doing the work, it would appear that the method of estimating the plumpness index on a scale of 1–10 would be the most useful. When the plumpness of a limited number of small samples is to be determined, and an accurate note is desired, the weight per measured bushel, as determined by the 4 cc. graduate, would appear to be the most useful. This method should be especially useful in experiments dealing with small samples harvested from pot cultures, in nutritional and disease studies as well as in plant breeding material. Weight per thousand kernels lacks reliability in comparisons when varieties and strains of different kernel size are involved, and is too laborious and expensive when thousands of determinations are to be made.

References

1. Bridgford, R. O. and Hayes, H. K. Correlation of factors affecting yield in hard red spring wheat. J. Am. Soc. Agron. 23 : 106-117. 1931.

2. Clark, J. A. and Smith, R. W. Inheritance in Nodak and Kahla durum wheat crosses for rust resistance, yield and quality at Dickinson, North Dakota. J. Am. Soc. Agron. 20 : 1297-1304. 1928.

3. Waldron, L. R. Yield and protein content of hard red spring wheat under conditions of high temperature and low moisture. J. Agr. Research, 47 : 129-147. 1933.

THE ESTIMATED NUMBER OF NEMAS IN THE SOILS OF MANITOBA[1]

By E. H. J. Marchant[2]

Abstract

Samples of soil, taken from 29 different sections of the arable portions of Manitoba, have been analyzed. Twelve species of eight genera of nematodes have been identified. No species of the genus *Heterodera* were found.

An endeavor has been made, first, to determine the approximate degree of infestation, and second, to correlate such factors as hydrogen ion concentration, moisture equivalent, and organic matter content with the nema counts.

The nematode population is somewhat higher in the soils of Manitoba than in those of many parts of the United States, but considerably lower than in those of North China.

The number of species seems to be limited.

The degree of infestation appears to be negatively affected by either hydrogen ion concentration or moisture equivalent, but is decidedly influenced by organic matter content.

Introduction

Despite the fact that the soil Nematoda are almost world-wide in distribution, and that they are of both economic importance and scientific interest, they have attracted the attention of comparatively few investigators. So far as the author has been able to ascertain, the nemas inhabiting Manitoba soils have, up to the present, remained unstudied.

In determining the importance of the soil nematodes, their numerical significance must first be ascertained. The figures obtained are considered by most authors to be a minimum, due to the fact that, among the many difficulties experienced in compiling a census, complete isolation is, as yet, technically impossible, and secondly, to the uneven distribution of the nematodes over a wide area. (Steiner (17).)

The samples of soil, cultivated and uncultivated, taken from 29 districts of the arable portions of Manitoba, for this survey, were of the following genetic types:—meadow-prairie, podsol, reclaimed-meadow, podsolic chernozem, degraded chernozem, meadow, peat, sandy chernozem, alkalinized meadow-prairie, river terrace (immature), woodland invasion of prairie, and river flood plain (immature)

Technique

All samples were taken at a uniform depth of 6 in. (15.2 cm.) and obtained by the use of a metal cylinder, having an internal diameter of 72.1 mm. (one one-millionth of an acre (25)), and a length of 12 in. Four samples were collected from each area, and the final census, in each case, was determined by averaging these.

[1] *Contribution from the Department of Zoology, University of Manitoba, Winnipeg, Manitoba, Canada. This paper is constructed from a thesis presented to the Committee on Postgraduate Studies, University of Manitoba, April, 1934, in partial fulfilment of the requirements for the M.Sc. degree.*

[2] *Research student.*

Isolation of the nematodes was accomplished by means of the Baerman apparatus, as used by Brown (4, 5) and described by Cort (9), with slight modifications. They were counted into a receptacle containing clean distilled water.

For the purpose of identification it was found best to examine the living organisms. However, when this was inconvenient, the following methods of fixing and preserving gave satisfactory results.

The specimens were relaxed and killed by heating in a hollow-ground slide containing a few drops of distilled water. Fixing was carried out by the use of Ditlevson's fixative, having the following composition: 6 parts formalin, 40 parts distilled water, 20 parts 90% alcohol, and 1 part acetic acid. From this the nematodes were transferred to a preservative composed of 68% of distilled water, 30% of 90% alcohol, and 2% of glycerin, in which they were left, unsealed, until water and alcohol had evaporated, leaving them finally in a slightly watery glycerin.

Table I shows the approximate number of nemas in the top 6 in. of an acre in each of the various soil types.

TABLE I

APPROXIMATE NUMBER OF NEMAS IN THE VARIOUS SOIL TYPES

Sample No.	Soil type	Soil texture	Approximate No. in the top 6 in. of an acre
1	Barnyard	Clay	6,250,500,000
2	Degraded chernozem	Silty clay	970,100,000
3	Chernozem	Silty clay	1,990,600,000
4	Garden	Silty clay loam	607,900,000
5	River terrace	Fine sandy loam	281,200,000
6	Meadow-prairie	Silty clay	4,980,000,000
7	Podzol	Fine sand	145,100,000
8	Reclaimed meadow	Silt loam	400,600,000
9	Podzol	Silty clay	878,900,000
10	Woodland invasion of prairie	Fine sandy loam	580,100,000
11	Podzolic	Silty clay loam	460,300,000
12	Chernozem	Heavy silty clay	634,600,000
13	Degraded chernozem	Clay	890,500,000
14	Garden	Silty clay	435,400,000
15	Peat	Muck	8,800,000,000
16	Degraded chernozem	Clay loam	2,200,000,000
17	Meadow	Silty clay loam	1,395,000,000
18	Degraded chernozem	H.V.F. sandy loam	643,500,000
19	Alkalinized meadow-prairie	Clay	480,600,000
20	Recent deposit	Silty clay	226,500,000
21	Meadow	Clay	262,800,000
22	Immature soil	Fine sandy loam	127,300,000
23	Sandy chernozem	L.V.F. sand	252,700,000
24	Chernozem	Fine sandy loam	325,100,000
25	Meadow-prairie	Clay	308,100,000
26	River flood plain	Silty clay loam	291,700,000
27	Chernozem	Silt loam	562,400,000
28	Meadow-prairie	Clay	2,790,000,000
29	Peat	Carr	*2,450

**One sample of 100 gm. only.*

TABLE II

MINIMUM NUMBER OF NEMAS PER ACRE IN VARIOUS FIELDS IN THE UNITED STATES

	Minimum number of nematodes per acre, top 6 in.
From Missouri corn field	648,000,000
North Carolina field	242,400,000
New Jersey field	129,600,000
Rhode Island field	610,800,000
New Hampshire field	99,600,000
Minnesota field	121,200,000
Vermont field	580,000,000
Kansas field	278,400,000

A census by Cobb (8) of fields in several of the United States is given in Table II, and is of interest for comparative purposes.

The results obtained would seem to indicate that the nematode population is somewhat higher, in the soils of Manitoba, than in those of many parts of the United States, but considerably lower than in those of North China (4, 5).

Taxonomy

The identification of the nematodes was facilitated by referring to descriptions or illustrations by the following authors: Baylis and Daubney (1), Cobb (6, 7, 8), Imms (10), Imperial Bureau of Agricultural Parasitology (11, 12), Micoletzky (13), Potts (14), Russell (15), Steiner (17, 18, 19, 20), Thorne (21, 22, 23, 24) and Waksman (25).

This survey yielded 12 species of 8 genera, and since they have all been previously described in detail by other investigators, the author has considered it necessary only to classify the species briefly, as follows:—

Nematoda

Fam. Rhabditidae
- Subfam. Rhabditinae
  - Gen. *Rhabditis* (Dujardin)
    - *R. elegans* (Maupas)
    - *R. cylindrica* (Cobb)
  - Gen. *Cephalobus* (Bastian)
    - *C. subelongatus* (Cobb)
    - *C. oxyuriodes* (DeMan)
  - Gen. *Acrobeles* (Von Linstow)
    - *A. cervus* (Thorne)
- Subfam. Cylindrolaiminae
  - Gen. *Plectus* (Bastian)
    - *P. cirratus* (Bastian)

Fam. Anguillulinidae
- Subfam. Anguillulinae
  - Gen. *Anguillulina* (Gervais and Van Beneden) (Syn. *Tylenchus*)
    - *A. pratensis* (DeMan)
  - Gen. *Ogma* (Southern) (Syn. *Hopolaimus*, Daday) (Syn. *Iota*, Cobb)
    - *O. octangulare* (Cobb)

Fam. Dorylaimidae
  Subfam. Dorylaiminae
    Gen. *Dorylaimus* (Dujardin) — *D. regius* (DeMan); *D. obtusicaudatus* (Bastian)

Fam. Oncholaimidae
  Subfam. Oncholaiminae
    Gen. *Mononchus* (Bastian) — *M. papillatus* (Bastian); *M. parvus* (DeMan)

Table III shows a list of the species and the various types of soil in which they were found.

TABLE III

SPECIES FOUND IN VARIOUS SOIL TYPES NUMBERED AS FOR TABLE I

Species	Sample number																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Acrobeles cervus							*										*						*						
Mononchus papillatus													*		*							*							*
Dorylaimus regius		*	*		*	*	*	*	*	*	*		*	*	*	*	*	*		*	*	*	*	*	*	*	*		*
Dorylaimus obtusicaudatus		*	*		*		*		*		*		*	*	*		*					*	*	*			*		
Rhabditis elegans	*			*			*																				*	*	
Ogma octangulare																							*						
Cephalobus subelongatus	*	*	*	*					*			*		*	*			*	*			*	*			*	*		*
Rhabditis cylindrica	*														*							*							
Mononchus parvus							*																						
Cephalobus oxyuriodes	*			*	*							*		*	*							*							*
Anguillulina pratensis		*			*					*															*				
Plectus cirratus			*				*									*													

It is interesting to note (Table III) that, of the twelve species isolated, three, *Dorylaimus regius*, *Dorylaimus obtusicaudatus*, and *Cephalobus subelongatus*, occur much more frequently than do any of the others; also that two of the species, *Ogma octangulare*, and *Mononchus parvus*, each occur only once, the former in a grassland soil (sandy chernozem), the latter in a woodland soil (podsol).

The Relations Between Degree of Infestation and Moisture Equivalent, Organic Matter Content, and pH Value of the Soil

The soils used for these correlations were taken from the same sections as were those of the census. All determinations were made in duplicate and

averaged. Table IV lists the values obtained for moisture equivalent, organic matter, and pH of each of the soil types, numbered as in Table I.

TABLE IV

pH, ORGANIC MATTER, AND MOISTURE EQUIVALENT VALUES OF EACH OF THE SOIL TYPES

Sample No.	pH		Organic matter, %	Moisture equivalent, %	Sample No.	pH		Organic matter, %	Moisture equivalent, %
	H_2O	KCl				H_2O	KCl		
1	8.19	7.44	21.70	46.20	16	8.13	6.94	14.37	33.74
2	7.43	6.44	11.30	41.35	17	8.23	7.14	12.23	32.07
3	6.56	6.03	12.40	42.11	18	7.07	6.36	9.11	34.84
4	7.99	7.02	9.70	33.76	19	7.77	6.65	11.53	39.96
5	8.39	7.19	4.60	13.96	20	7.68	6.89	3.18	41.81
6	7.87	7.15	21.20	41.44	21	8.08	6.77	3.70	46.25
7	7.75	6.97	2.21	5.40	22	7.28	6.35	90.13	106.79
8	6.82	6.04	6.20	24.60	23	6.19	6.13	3.14	16.51
9	6.33	5.66	11.43	38.94	24	7.78	6.86	3.53	9.80
10	7.66	6.85	8.05	21.68	25	7.33	6.36	5.90	20.62
11	7.44	6.69	6.70	28.47	26	7.78	7.05	5.70	38.86
12	8.41	7.17	8.74	35.79	27	7.60	6.70	7.62	25.95
13	6.67	6.06	10.70	46.23	28	8.39	7.39	5.13	30.15
14	7.92	7.06	6.40	32.54	29	7.29	6.37	17.81	44.62
15	6.10	5.70	38.83	90.87					

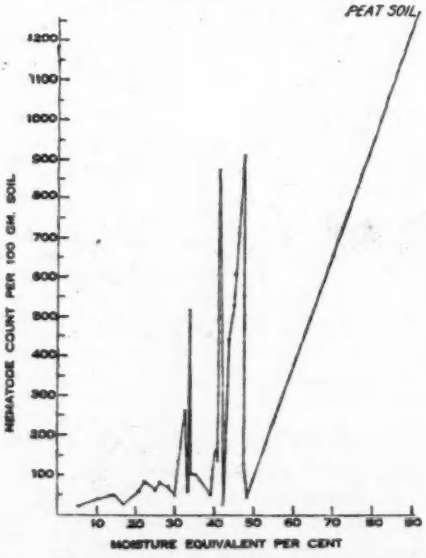


FIG. 1. *Relation between nema count and moisture equivalent.*

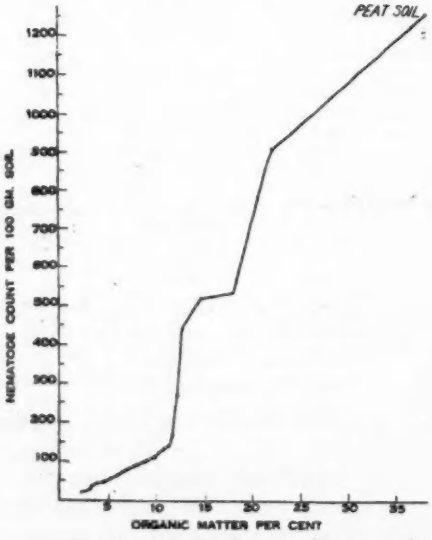


FIG. 2. *Relation between nema count and organic matter.*

Moisture Equivalent (M.E.)

This value was determined by the method described by Briggs and McLane (3). The percentage of moisture lost was determined on a dry soil basis; the percentage loss being the M.E. The relation between nema counts and moisture equivalent is shown in Fig. 1.

Organic Matter

This was determined volumetrically, by means of chromic acid (Schollenberger (16)). The percentage of organic matter was calculated on the basis of the dry weight of the soil. Fig. 2 shows the relation between organic matter and nematode count.

Hydrogen ion Concentration

The pH of the soils was determined, electrometrically, both in distilled water, and in normal potassium chloride solution, by means of the quinhydrone electrode (Biilman and Tovborg-Jensen (2)). The relation between pH and nema count is shown in Fig. 3.

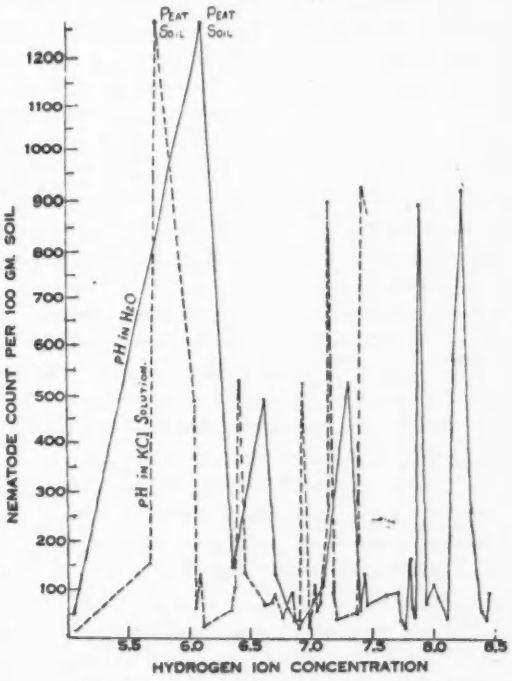


FIG. 3. *Relation between nema count and pH.*

Discussion

The results of this survey would indicate that while the nematode infestation is somewhat heavy, the number of species is limited. Extreme variations in temperature experienced in this province may perhaps be a contributory cause. This, however, can only be determined after the analysis of samples of soil, of a restricted area, taken at frequent intervals, and stratigraphically, over a period of one or more years.

Moisture equivalent (M.E.) appears to be a negative factor in affecting the nema population, although there seems to be a slight correlation below 15%. Above this the correlations are contradictory, except in the case of the peat soil. The writer is of the opinion that, in this instance, it is the high organic matter content which is the determining factor and not the M.E.

The organic matter content varied from 2–38% and appears to be closely related to the nematode counts, irrespective of soil type.

The hydrogen ion concentration (pH) of Manitoba soils, generally speaking, ranges from 5.50–8.00. Within these limits, no correlation with the number of organisms is apparent.

The fact that no species of *Heterodera* were isolated is not surprising, for in no country do they seem to be widespread, but rather confined to restricted areas. As far as the author has been able to ascertain, infestation has occurred only in two small districts, one near Chatham, Ontario, where *Heterodera*

schachtii Schmidt was found in a sugar beet field, the other near Humboldt, Saskatchewan, where a few wheat fields were mildly infected by a new species, *Heterodera punctata* Thorne. The writer obtained, through the kindness of Mr. R. C. Russell, Plant Pathologist, Dominion Laboratory of Plant Pathology, Saskatchewan, some specimens of preserved and dried wheat roots infected with the *H. punctata* Thorne. On these roots were a number of cysts, both in the white and in the brown or preservation stages. These were found to be pear-shaped rather than lemon-shaped as in the case of *Heterodera schachtii* Schmidt. Other differentiating characteristics of *H. punctata* Thorne, are that the female is smaller than that of *H. schachtii* Schmidt, and that it possesses a punctate cuticle.

Mr. G. Thorne is of the opinion that the *punctata* form of *Heterodera* is a native parasite of some indigenous plant or plants, and from the fact that it has selected wheat as its host, it seems probable that one or more of the native grasses will be found to be the original host.

No methods that are economically feasible have as yet been devised for the total eradication of *Heterodera* species. Up to the present, only crop rotation gives any worthwhile results. Stringent measures must also be taken to prevent the spread of infection. In the case of *H. punctata* Thorne, a system of crop rotation cannot be planned until its original host has been definitely determined.

During the period of this investigation, the crops, generally speaking, were in a poor condition as a result of drought and grasshopper injury, thus making it impossible to form any opinion as to the effect which the nematodes might have had upon their growth. Their effect on soil fertility is not yet fully known.

For the purpose of observing the predatory feeding habits of *Mononchus papillatus* a few experiments were carried out in the laboratory by feeding both larval and adult specimens of other species of nematodes. In every instance the adults were able to throw off the *Mononchus*, but in the case of the larvae, the body contents were first sucked out, and the cuticle then swallowed, with one exception. In this case a Rhabdite larva was swallowed whole. After being kept for ten weeks in water containing a few minute particles of soil, protozoa and other micro-organisms, but no nemas, they showed no signs of excessive hunger when offered larvae.

Many of the nematodes encyst whenever soil conditions become unfavorable, and in some instances, after having remained in this state for ten and even twenty-five years, they have become active again, when suitable conditions were restored. The author obtained from the Soils Department, University of Manitoba, samples of soil which had stood in the laboratory from ten to twelve years. Analysis of these however, gave negative results.

Acknowledgments

The author is greatly indebted to Prof. R. A. Wardle, under whose direction this investigation was carried out, and also to the following, who have assisted

with advice, literature, and specimens: Prof. J. H. Ellis, Soils Department, University of Manitoba, Dr. G. Steiner, Senior Nematologist, U.S. Dept. of Agriculture, Mr. G. Thorne, Associate Nematologist, Dr. H. D. Brown, Head of the Research Dept., Canada and Dominion Sugar Co., Dr. J. N. Oldham, Deputy Director, Imperial Bureau of Agricultural Parasitology, and Mr. R. C. Russell, Plant Pathologist, Dominion Laboratory of Plant Pathology, Saskatchewan.

References

1. Baylis, H. A. and Daubney, R. A synopsis of the families and genera of nematodes. London. 277 p. 1926.
2. Biilman, E. and Tovborg-Jensen, S. On the determination of the reaction of soils by means of the quinhydrone electrode. Trans. 2nd Congress Int. Soc. Soil Sci. Groningen (Holland). Vol. B. 236-274. 1927.
3. Briggs, L. J. and McLane, J. W. Moisture equivalent determinations and their applications. Proc. Am. Soc. Agron. 2, p. 139. 1910.
4. Brown, H. D. Nematodes in the soil. Lingnan Sci. J. (China) 8 : 11-24. 1929.
5. Brown, H. D. Sugar beet Nematode—*Heterodera schachtii*—a new parasite in Canada. Sci. Agr. 12 : 544-552. 1932.
6. Cobb, N. A. Nematodes and their relationships. Yearbook, U.S. Dept. Agr. Washington. pp. 457-490. 1914.
7. Cobb, N. A. Freshwater biology. Ward and Wipple. pp. 459-535. 1918.
8. Cobb, N. A. *Mononchus*: a genus of free-living predatory nematodes. Soil Sci. 3 : 431-486. 1917.
9. Cort, W. W. Investigations on the control of hookworm disease. Part II. The description of an apparatus for isolating infective hookworm larvae from the soil. Am. J. Hyg. 2 : 1-16. 1922.
10. Imms, A. D. Micro-organisms of the soil. E. J. Russell *et al.* Longmans Green and Co., London. p. 147-163. 1923.
11. Imperial Bureau of Agricultural Parasitology. Differential diagnosis of plant parasitic eelworms. No. 5. pp. 1-12. 1932.
12. Imperial Bureau of Agricultural Parasitology. Potato sickness and the eelworm *Heterodera schachtii*. No. 6. pp. 1-22. 1932.
13. Micoletzky, H. Die Freilebenden Erd-Nematoden. Arch. Naturgesch. Abt. A, Jahrg. 87 Heft 8-9. pp. 1-650. 1922.
14. Potts, F. A. Notes on free-living nematodes. Quart. J. Micro. Soc. 55 : 433-484. 1910.
15. Russell, E. J. Soil conditions and plant growth. Longmans Green and Co., London. pp. 411-413. 1932.
16. Schollenberger, C. J. Volumetric method of determining soil organic matter by means of chromic acid. Soil Sci. 24 : 65-68. 1927.
17. Steiner, G. The nemic population of the soil. Proc. First Int. Soc. Soil Sci. Congress, 4. pp. 360-366. 1928.
18. Steiner, G. and Heinly, H. The possibility of control of *Heterodera radicicola* and other plant injurious nemas by means of predatory nemas, especially by *Mononchus papillatus* bastian. J. Wash. Acad. Sci. 12 : 367-386. 1922.
19. Steiner, G. On some plant parasitic nemas and related forms. J. Agr. Research, Wash. 28 : 1059-1066. 1924.
20. Steiner, G. *Tylenchus pratensis* and various other nemas. J. Agr. Research, Wash. 35 : 961-981. 1927.
21. Thorne, G. Utah nemas of the genus *Mononchus*. Trans. Am. Micro. Soc. 43 : 157-171. 1924.
22. Thorne, G. The genus *Acrobeles* von Linstow, 1877. Trans. Am. Micro. Soc. 44 : 171-210. 1925.
23. Thorne, G. The life history, habits, and economic importance of some *Mononchs*. J. Agr. Research, Wash. 34 : 265-286. 1927.
24. Thorne, G. Nematodes from the summit of Long's Peak, Colorado. Trans. Am. Micro. Soc. 48 : 181-195. 1929.
25. Waksman, S. A. Principles of soil microbiology. Williams and Wilkins, Baltimore. pp. 431-451. 1932.

schachtii Schmidt was found in a sugar beet field, the other near Humboldt, Saskatchewan, where a few wheat fields were mildly infected by a new species, *Heterodera punctata* Thorne. The writer obtained, through the kindness of Mr. R. C. Russell, Plant Pathologist, Dominion Laboratory of Plant Pathology, Saskatchewan, some specimens of preserved and dried wheat roots infected with the *H. punctata* Thorne. On these roots were a number of cysts, both in the white and in the brown or preservation stages. These were found to be pear-shaped rather than lemon-shaped as in the case of *Heterodera schachtii* Schmidt. Other differentiating characteristics of *H. punctata* Thorne, are that the female is smaller than that of *H. schachtii* Schmidt, and that it possesses a punctate cuticle.

Mr. G. Thorne is of the opinion that the *punctata* form of *Heterodera* is a native parasite of some indigenous plant or plants, and from the fact that it has selected wheat as its host, it seems probable that one or more of the native grasses will be found to be the original host.

No methods that are economically feasible have as yet been devised for the total eradication of *Heterodera* species. Up to the present, only crop rotation gives any worthwhile results. Stringent measures must also be taken to prevent the spread of infection. In the case of *H. punctata* Thorne, a system of crop rotation cannot be planned until its original host has been definitely determined.

During the period of this investigation, the crops, generally speaking, were in a poor condition as a result of drought and grasshopper injury, thus making it impossible to form any opinion as to the effect which the nematodes might have had upon their growth. Their effect on soil fertility is not yet fully known.

For the purpose of observing the predatory feeding habits of *Mononchus papillatus* a few experiments were carried out in the laboratory by feeding both larval and adult specimens of other species of nematodes. In every instance the adults were able to throw off the *Mononchus*, but in the case of the larvae, the body contents were first sucked out, and the cuticle then swallowed, with one exception. In this case a Rhabdite larva was swallowed whole. After being kept for ten weeks in water containing a few minute particles of soil, protozoa and other micro-organisms, but no nemas, they showed no signs of excessive hunger when offered larvae.

Many of the nematodes encyst whenever soil conditions become unfavorable, and in some instances, after having remained in this state for ten and even twenty-five years, they have become active again, when suitable conditions were restored. The author obtained from the Soils Department, University of Manitoba, samples of soil which had stood in the laboratory from ten to twelve years. Analysis of these however, gave negative results.

Acknowledgments

The author is greatly indebted to Prof. R. A. Wardle, under whose direction this investigation was carried out, and also to the following, who have assisted

with advice, literature, and specimens: Prof. J. H. Ellis, Soils Department, University of Manitoba, Dr. G. Steiner, Senior Nematologist, U.S. Dept. of Agriculture, Mr. G. Thorne, Associate Nematologist, Dr. H. D. Brown, Head of the Research Dept., Canada and Dominion Sugar Co., Dr. J. N. Oldham, Deputy Director, Imperial Bureau of Agricultural Parasitology, and Mr. R. C. Russell, Plant Pathologist, Dominion Laboratory of Plant Pathology, Saskatchewan.

References

1. Baylis, H. A. and Daubney, R. A synopsis of the families and genera of nematodes. London. 277 p. 1926.
2. Biilman, E. and Tovborg-Jensen, S. On the determination of the reaction of soils by means of the quinhydrone electrode. Trans. 2nd Congress Int. Soc. Soil Sci. Groningen (Holland). Vol. B. 236-274. 1927.
3. Briggs, L. J. and McLane, J. W. Moisture equivalent determinations and their applications. Proc. Am. Soc. Agron. 2, p. 139. 1910.
4. Brown, H. D. Nematodes in the soil. Lingnan Sci. J. (China) 8 : 11-24. 1929.
5. Brown, H. D. Sugar beet Nematode—*Heterodera schachtii*—a new parasite in Canada. Sci. Agr. 12 : 544-552. 1932.
6. Cobb, N. A. Nematodes and their relationships. Yearbook, U.S. Dept. Agr. Washington. pp. 457-490. 1914.
7. Cobb, N. A. Freshwater biology. Ward and Wipple. pp. 459-535. 1918.
8. Cobb, N. A. *Mononchus*: a genus of free-living predatory nematodes. Soil Sci. 3 : 431-486. 1917.
9. Cort, W. W. Investigations on the control of hookworm disease. Part II. The description of an apparatus for isolating infective hookworm larvae from the soil. Am. J. Hyg. 2 : 1-16. 1922.
10. Imms, A. D. Micro-organisms of the soil. E. J. Russell *et al.* Longmans Green and Co., London. p. 147-163. 1923.
11. Imperial Bureau of Agricultural Parasitology. Differential diagnosis of plant parasitic eelworms. No. 5. pp. 1-12. 1932.
12. Imperial Bureau of Agricultural Parasitology. Potato sickness and the eelworm *Heterodera schachtii*. No. 6. pp. 1-22. 1932.
13. Micoletzky, H. Die Freilebenden Erd-Nematoden. Arch. Naturgesch. Abt. A, Jahrg. 87 Heft 8-9. pp. 1-650. 1922.
14. Potts, F. A. Notes on free-living nematodes. Quart. J. Micro. Soc. 55 : 433-484. 1910.
15. Russell, E. J. Soil conditions and plant growth. Longmans Green and Co., London. pp. 411-413. 1932.
16. Schollenberger, C. J. Volumetric method of determining soil organic matter by means of chromic acid. Soil Sci. 24 : 65-68. 1927.
17. Steiner, G. The nemic population of the soil. Proc. First Int. Soc. Soil Sci. Congress, 4. pp. 360-366. 1928.
18. Steiner, G. and Heinly, H. The possibility of control of *Heterodera radicicola* and other plant injurious nemas by means of predatory nemas, especially by *Mononchus papillatus* bastian. J. Wash. Acad. Sci. 12 : 367-386. 1922.
19. Steiner, G. On some plant parasitic nemas and related forms. J. Agr. Research, Wash. 28 : 1059-1066. 1924.
20. Steiner, G. *Tylenchus pratensis* and various other nemas. J. Agr. Research, Wash. 35 : 961-981. 1927.
21. Thorne, G. Utah nemas of the genus *Mononchus*. Trans. Am. Micro. Soc. 43 : 157-171. 1924.
22. Thorne, G. The genus *Acrobeles* von Linstow, 1877. Trans. Am. Micro. Soc. 44 : 171-210. 1925.
23. Thorne, G. The life history, habits, and economic importance of some *Mononchs*. J. Agr. Research, Wash. 34 : 265-286. 1927.
24. Thorne, G. Nematodes from the summit of Long's Peak, Colorado. Trans. Am. Micro. Soc. 48 : 181-195. 1929.
25. Waksman, S. A. Principles of soil microbiology. Williams and Wilkins, Baltimore. pp. 431-451. 1932.

THE RENEWAL AND REPLACEMENT OF THE STYLETS OF SUCKING INSECTS DURING EACH STADIUM, AND THE METHOD OF PENETRATION[1]

By A. D. Heriot[2]

Abstract

In a study of the mouth parts of the scale and aphis, certain conclusions corresponding to those of Hermann Weber were independently drawn. While several phases of the subject need clarifying, it is definitely established that the retort-shaped organs at the bases of the stylets are masses of hypodermal cells constituting deep invaginations of the integument. Within these invaginations, which lengthen out in a circular manner during each stadium, new stylets are built up for each successive instar. In those members of the Hemiptera in which the head is deflected, the four stylets are separately coiled in the cephalic region and at a definite stage during each ecdysis the new stylets pass down to take the place of the old which are discarded at the moult.

The manner of this renewal and replacement of the stylets appears to be, in many respects, unique. But, inasmuch as the stylets are simple hollow chitinous structures, they may be said histologically to bear a closer resemblance to other cuticular processes than they do to the more complex organs arising from imaginal buds during metamorphosis. An analogy to the renewal of the stylets is presented in the development of the spines and hairs of the body. The methods whereby the coiled stylets are expelled from the head are discussed.

The action of the protractor muscles in the aphis and scale is altogether inadequate to explain the means of penetration into the compact tissues of woody growths. More especially is this the case where the stylets are much longer than the labium, and are, moreover, looped within the body and thus neutralize the effect of muscular action at the base. New facts are advanced to supplement those already known, and it is submitted that the stylets are propelled forward by successive short holds contrived by the joint action of the labrum and labium. In the aphis the main function of the stylet muscles is directional control of the tips, enabling a short stylet to explore a large area. The absence of this control in the scale is compensated for by the provision of longer stylets. Directional control permits of selective feeding, giving rise to a sense of taste. This is manifest in specific types of injury to the host plant and also by a modification in the insect of accessory structures of the mouth.

Introduction

In the spring of 1928, investigations were commenced in the Entomological Laboratory at Vernon, British Columbia, to determine the part played by the woolly aphis of apple in facilitating the spread of canker. These studies were pursued over a period of several years, and the morphology and mechanism of the mouth parts of the aphis naturally received considerable attention.

The mouth parts of the woolly aphis had already undergone considerable study by other workers, most notably by J. Davidson (3) in England and A. C. Baker (1) in the U.S.A. In fact, there is no aphis on which there is a more extensive literature, with regard to both its external and internal anatomy, than *Eriosoma lanigerum* Haus. Nevertheless, such interesting features as the renewal and replacement of the stylets at the moult and the method whereby these delicate structures are inserted into the hard tissues of the host-plant have until recently been barely touched upon.

[1] *Original manuscript received May 3, 1934.*
Contribution from Dominion Entomological Branch, Department of Agriculture, Ottawa, Canada.

[2] *Insect Pest Investigator, Dominion Entomological Laboratory, Vernon, British Columbia.*

Considerable information was gathered on these points in 1929 and 1930 when, in addition to the aphis, the scales and leaf-hoppers with similar deflected heads were examined for the purpose of obtaining comparative data. It was discovered that at each moult a new and more efficient set of stylets, which previously were coiled within the head, became available. This fact was alluded to by the writer (6) in a paper read before the British Columbia Entomological Society, and aroused considerable interest, being regarded as something new and therefore requiring further substantiation than could at that time be provided.

In the previous year, 1930, Hermann Weber's "Biologie der Hemipteren", published in Berlin, demonstrated, with much original illustration, the same interesting point and extended its application to the whole of the Hemiptera. While Weber is referred to by Imms (8) in his "Recent Advances in Entomology" (1931) it is evident that, when discussing the cephalic appendages, Imms had no knowledge of the "Biologie der Hemipteren" which deals so effectively with recent discoveries on this point.

This remarkable monograph came before the writer in June, 1933, through the courtesy of Prof. G. J. Spencer of the University of British Columbia, who had taken a great interest in another paper which was read before the Society, March, 1933, and which is now presented in a revised form.

I. Renewal and Replacement of the Stylets during each Stadium

In his text book, 1930, Imms (7) on summing up what was then known with regard to the mouth parts of the Hemiptera, refers to the oval areas of tissue at the enlarged proximal ends of both pairs of stylets as "the retort-shaped organs whose function is problematical" (Fig. 1). Davidson (2) was probably the first to draw attention to these peculiar organs in the embryo of *Eriosoma lanigerum* Haus. without appreciating their significance.

It is now established that these "retort-shaped organs" are masses of hypodermal cells which are engaged in building up new stylets to replace in due time the stylets in use which will subsequently be discarded at the moult.

In examining the mouth parts it was very soon found that the scale insects lend themselves more readily to microscopic study than the aphids. The scales such as *Aspidiotus ostreaeformis* Curtis are very thin and transparent. They are unable to move out of the field of vision under a high power when examined alive, and their mouth parts can thus be observed in action.

The particular problems that this paper endeavors to elucidate have always been regarded as of peculiar and special interest in the biology of the scale insects. Imms (7) sets out the nature of the problem as follows: "In Coccidae, the rostrum (labium) is very short and the stylets extremely long and the mechanism by which the latter are inserted into the plant and afterwards withdrawn and looped within the body is difficult to conceive." We are not concerned at the moment with the method of insertion, which will be dealt

with later, but more particularly with the idea of a withdrawal of the stylets which is conveyed in this sentence. This implies that the accepted opinion arrived at in 1930 was that the same set of stylets served the insect throughout the course of its three stadiums. There are several reasons for this idea. First, there is the inordinate and, to all appearances, unnecessary length of the stylets of the nymph which will be discussed later. Second, the fact that the stylets are never found with the exoskeleton of the other mouth parts attached to the cast skin, as in the case of the other Homoptera, and finally, there is the ease with which the scales can be lifted from the bark at the time of moulting (4). The last is entirely due to the fact that the old stylets embedded in the bark are more easily separated from the insect at the approach of the moult than they are from the host plant.

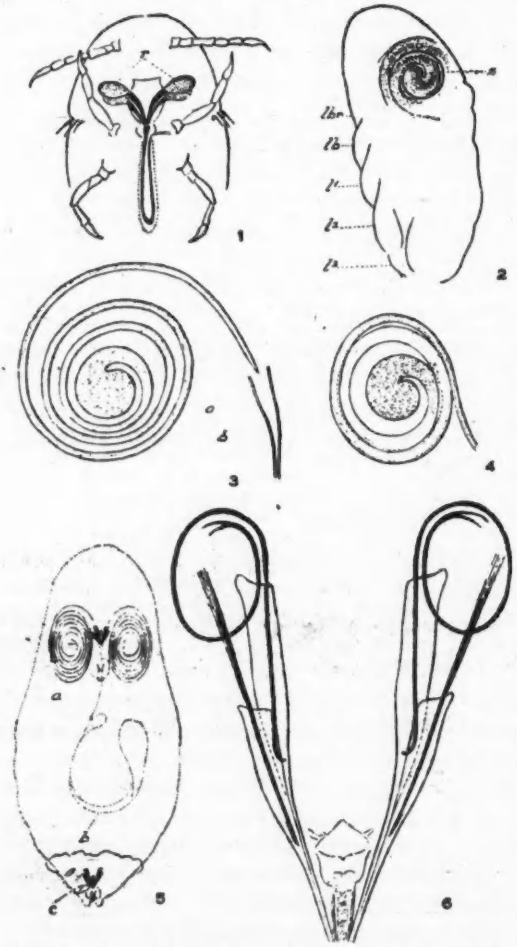


FIG. 1. *Coccus hesperidum. Figure adapted from Berlese and given by Imms.* r, *retort-shaped organs.* FIG. 2. *Section through embryo of Eriosoma lanigera.* s, *coiled stylet;* lbr, *labrum;* lb, *labium;* l 1-3, *legs.* FIG. 3. *Coiling stylet of Aspidiotus ostreaeformis from section.* a, *new stylet;* b, *old stylet.* FIG. 4. *Stylet coil dissected out of Eriosoma lanigera.* FIG. 5. *Lepidosaphes ulmi at second moult.* a, *stylets before moult;* b, *stylets after moult;* c, *moulted mouth parts in crumpled skin at posterior end of exuvia.* FIG. 6. *New and old stylets of Typhlocyba rosae showing coiled maxillary and straight mandibular stylets.*

Our observations certify that no withdrawal of any kind takes place once the stylets are inserted into the bark. The old stylets are broken off during the struggles incurred in moulting. Sections through the bark of scale-fed twigs show the cast-off stylets of the two instars and the adult in the relative positions that these would be expected to assume when allowance is made for the

slight movement that accompanies the insect's efforts to free itself from the exuvia. The stylets of each instar can readily be distinguished in these sections both by their respective diameters and by the different depths of their penetration.

Since these broad facts were known in 1929, considerable evidence has accumulated which allows for a more detailed description of what actually occurs at birth and at the moult with regard to the building up, renewal, and replacement of the stylets.

An examination of the embryo of any aphis or scale, shortly before birth, will show the long maxillary and mandibular stylets separately coiled in the head as in Fig. 2. During parturition or emergence from the egg, as the case may be, the separate stylets travel down from the head, their points converging to meet at the base of the labrum. Here the stylets coalesce and thence proceed as a single piercing and sucking organ to enter the labium.

The labium of the aphis is generally sufficiently long to accommodate the length of the stylets. In the scale, however, this is not the case, and only a small part of the stylets can find room in the very abbreviated labium. Hence all except the tips of the long stylets are, as Imms (7) expresses it, "looped and coiled upon themselves into the backwardly directed pocket (the crumena)."

This passing of the coiled stylets in the head to the labium and then to the crumena always takes place in *Lepidosaphes ulmi* L. at a definite stage of eclosion from the egg. This occurs before the head has freed itself from the egg envelope. Transitional stages are difficult to procure because, when once started, the movement seems to be very rapid. Only among large numbers of hatching nymphs have cases been observed with part of the stylets still in the head and part beginning to loop in the crumena. In the majority of cases, where the normal process is checked by examination media, the whole of the stylets pass out prematurely through the labium instead of folding back into the pocket provided for them.

When the stylets are in place, the invagination of hypodermal cells which brought them into being shrinks into a more or less oval mass of tissue around the bases of the stylets in the head, forming the retort-shaped organs. A small fold of this tissue extends like the finger of a glove into the mouth of each retort. At the apex of this fold the tip of the new stylet for the second instar begins to form. As the new stylet is laid down from the tip, the oval mass of tissue bunched at the mouth of the retort takes on a tubular form and lengthens out in a circular manner to form subsequently a coil resembling the curled frond of a fern (Fig. 3). In the core of this curling stem the new stylet is gradually built up, its relative proportions to the surrounding tissue of hypodermal cells being much the same as those of the lead to the wood of a cedar pencil.

As the long stylets proceed to be built up from the tip, the surrounding tissue retreats further and further towards the unfinished proximal end, increasing the number of convolutions in the coil, as it manufactures the

stylet. On completion of the requisite length, the creative cells bunch up at its base, later to insert another finger-like fold into the mouth of the new retort in the centre of the coil. This small fold, in turn, will subsequently furnish the adult with its stylet.

The scale has two or three weeks in which to lay down the long stylets for the succeeding instar in the manner described. On the other hand the aphis with its much shorter stylets accomplishes this feat in three or four days (Fig. 4). In *Typhlocyba rosae*, where the maxillary are longer than the mandibular stylets, the former are coiled while the latter find plenty of room in the buccal cavity of the head to develop without coiling (Fig. 6).

The new stylets of the second instar of *Lepidosaphes ulmi* L. are half as big again in both diameter and length as those of the first instar, which they supersede. At a definite stage of the moult, soon after the ventral skin has ruptured, the new stylets pass, as did those of the first instar, from the head to the body. The same quick transition from four separate coils in the head to a single sucking organ gracefully looped in the crumena attends the second and final moult (Fig. 5).

It may be of interest here to review briefly the steps that enquiry took in exploring this new ground. There was first the early discovery that the stylets of the scale were invariably coiled in the head previous to the moult, which at first suggested that the stylets were withdrawn into the head while the crumena was being renewed. The fact was patent, however, that the stylets of both the aphids and leafhoppers were cast off with the moulted skin, and it seemed most unlikely that the scale should be an exception to so general a rule. The finding of the cast-off stylets in the bark and the further fact that the formation of new stylets could be discerned when the old mouth parts were still functioning, led to an examination of the aphis and leafhoppers. This revealed the same condition of coiled stylets within the head in various stages of development. The serrated tip of the mandibular stylet of *Typhlocyba rosae* L. could unmistakably be detected in the retort of the mandibular stylet in use (Fig. 6).

The question arose as to whether this development and replacement of the stylets was something unique, or whether this method was duplicated in other structures at the moult. As it was quite out of the question that such purely chitinous hollow structures, without any matrix, could grow and be moulted like the other appendages, it seemed likely that the stylets, which are sometimes called the feeding bristles or setae, might be renewed as are many of the cuticular hairs of the body. These also become greater in length at each moult. The tubercles of the new hairs arise beneath the tubercles of the old, but the new hair itself lies compressed beneath the old skin and becomes erect when the body has dried after moulting.

A glance at Fig. 7, showing a multicellular spinal outgrowth *a*, and a single-celled seta *b*, will permit of the conception of a multicellular setal ingrowth *c*, illustrating the formation of a stylet. In this illustration the multicellular spine would correspond to the formation of the mandible of a biting insect.

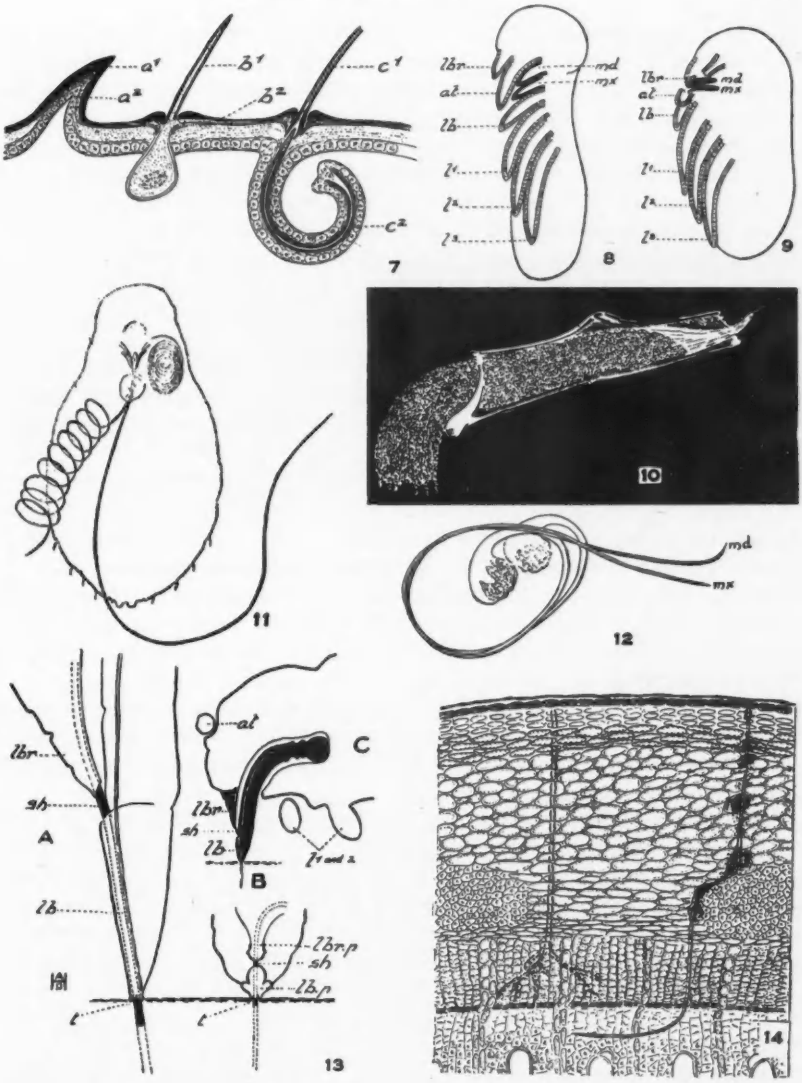


FIG. 7. a^1, a^2, *multicellular spinal outgrowth;* a^1, *old part,* a^2, *new part.* b^1, b^2, *unicellular setal outgrowth;* b^1, *old part,* b^2, *new part.* c^1, c^2, *multicellular setal ingrowth;* c^1, *old part,* c^2, *new part.* FIG. 8. *Embryo of biting insect. md, mandible; mx, maxilla; lbr, labrum at antenna; lb, labium;* l^{1-3}, *legs.* FIG. 9. *Embryo of scale; legend as in Fig. 8.* FIG. 10. *Broken stylet retort of Typhlocyba rosae showing tip of new stylet protruding beyond break with ligament.* FIG. 11. *Lepidosaphes ulmi; mandibular stylet issuing prematurely like a spring in corkscrew fashion.* FIG. 12. *Stylets of Lygus pratensis dissected from thorax.* FIG. 13. *Method of penetration. A, Eriosoma lanigera; B, Lepidosaphes ulmi; C, Eriosoma lanigera, section showing labium retracted into body. sh, short hold on stylet; t, thrust; lbr, labrum; lbr p, process at tip of labrum; at, antenna;* l^1 *and* l^2, *legs.* FIG. 14. *Stylet paths; that of Eriosoma lanigera on left, showing diversions by the tips and no injury to cortical cells; that of Lepidosaphes ulmi on right, showing absence of diversions and injury to cortical cells. Injury to phloem tissues not shown. (Diagrammatic in part.)*

Both the mixillary and mandibular stylets arise between the labrum and the labium as do their counterparts in biting insects. Fig. 8 shows the profile of the embryo of a biting insect with the outgrowths of maxilla and mandible, and Fig. 9 the profile of a scale embryo where the absence of projecting mouth parts must be accounted for by corresponding ingrowths.

Weber (9) gives a drawing, after Seidel, of the embryo of *Pyrrochoris apterus* Fall showing small projections which later become sunk into the head to form the first retorts out of which the stylets are built up. Observations with regard to the scale indicate that the long stylets are well advanced at the embryonic stage depicted by Seidel. When the embryo of the scale has gone through its reversion and the amnion has broken, the stylets are plainly visible. The writer cannot throw much light on the embryological development of the stylets except to maintain that the original invaginations must occur long before the reversion of the embryo, and in the deflected heads of the aphis and scale probably arise within a fold that separates the epipharynx from the hypopharynx.

There will be less cause for controversy in discussing the manner in which the stylets are drawn out or expelled from the head. Weber shows that the tips of the stylets are sometimes fastened to the inner side of the retort by a short ligament, and in the case of *Aradus* sp., to the extreme outer edge.

As the old stylets are cast off, the new stylets are drawn into place. This is corroborated in Fig. 10 which shows the broken retort of the maxilla of *Typhlocyba rosae* L. with a ligament at the tip of the new stylet protruding beyond the break.

No similar attachment has been discerned in *Eriosoma lanigerum* where the tips of the new stylets intrude into the retorts of the old, but as the old skin is cast away it is likely that a similar method of drawing out the stylets prevails.

In the case of the scale the skin is not cast away at the moult, the hard dorsal exuvia being only loosened and the ventral skin containing the mouth parts merely pushed down to its posterior end (Fig. 5). The new stylets, moreover, come down and are looped in the crumena soon after the ventral skin has broken at the anterior end. Some expelling force in this case seems to be essential. As a possible clue in this direction Fig. 11 illustrates a case where, in the course of mounting a moulting scale, the stylets have escaped prematurely through the labium instead of bending back into the crumena. One of the mandibular stylets is seen to have emerged in corkscrew fashion which suggests that the coiled stylets, as they mature, acquire the nature of a spring. In addition it can be shown that considerable pressure is exerted on the head by the narrowness of the exuvia. At an earlier stage the coils are conspicuously rounded, but at the approach of the moult they assume a distinctly oval form due to the compression of the head. Likewise the head of the young woolly aphis during parturition is held for a considerable time, and any pressure on the coils, which may at this stage have developed a tendency to straighten out, might initiate their expulsion. Once started, the labrum could then come into play in assisting their exit from the head.

As previously intimated, these studies have been confined to a few species where the head is deflected. Weber (9) shows that where the head is normal the stylets are coiled in the thorax. This is verified in Fig. 12, which shows the coiled stylets for the prospective adult dissected out of the thorax of the pupal instar of *Lygus pratensis* L.

II. Penetration of the Plant Tissues

The foregoing attempt to describe adequately the renewal and replacement of the stylets at the moult will assist in throwing light on the mechanical means whereby these slender structures are inserted into hard tissues.

Imms (7) says: "The problem which requires solution is the method by which long, slender and pointed stylets can be forced to the requisite depth into the tissues of a plant. In those cases where the stylets are but little longer than the labium, it has usually been explained that the action of the protractor muscles applied at the bases of the stylets, forces the latter into the plant, and that they are guided by the labrum and the grooved labium in their course."

No problem is presented in the case of the tarnished plant bug *Lygus pratensis* L. The penetration of soft tissues such as a bud seems to require no mechanism other than the bending of the labium as the head is lowered to force the stout stylets into the soft tissue. The same may be said of the leaf hopper, where the operation of penetrating the loose mesophyll of a leaf can be readily comprehended. In both these cases the mandibles are sharply serrated and with the muscles at their base no doubt play an important part in tearing out a path for the maxillae.

It is not such a simple matter to explain the penetration through the compact cortex of woody growths by the much more delicate stylets of the woolly aphis. Under a hand lens the newly born aphis may be observed sensing out a suitable spot to engage on, by means of the sensory organs at the tip of its long labium. The mandibular stylets are not serrated, and while there is proof that these can be used to deflect the tip of the maxillae in diverse directions after the stylets are inserted, their powers of penetration appear to be feeble. To make up for this deficiency the aphis is equipped with a comparatively much longer labrum than the tarnished plant bug and the leafhopper. This labrum is grooved along its length. A considerable portion of the stylet can thus be gripped with the whole weight of the head behind it. The long labium is also grooved and, moreover, can be retracted into the body, thus acting as a diminishing brace for the stylets. By successive short stages the labrum is brought into conjunction with the tip of the labium when the stylets are fully inserted.

When penetration by the scale is considered, an entirely different set of conditions has to be contended with. The scale stylets will not only penetrate the mature cortex of four- and five-year-old branches, but in new wood, where the layer of bark is thin, will enter the immature woody tissues. Cramped

beneath the shell-like exuvia the scale has little freedom of movement or choice of a suitable starting point and the tiny pimple of a labium is most inadequate as a brace. The muscles have little or no control over the tips of the stylets. This is accounted for by the long length of loosely looped stylets in the crumena. Even the possession of a labrum to make up for these deficiencies seems to have been denied the insect, according to other workers.

Evidently reasoning on somewhat similar lines Grove (5) and Berlese are referred to by Imms (7) as suggesting "that by means of blood pressure the apex of the labium becomes extended and consequently grips the stylets tightly after the manner of a pair of forceps. The projecting portions of the stylets would be forced a short distance into the plant tissues. This being accomplished, the internal pressure would be slackened, which would result in the grip being released and the labium would become slightly shorter, so that the apex would have traveled a short distance upwards along the stylets. The pressure being renewed, the grip is re-established and the stylets forced in a step further, and so on until the required amount of penetration may be accomplished."

This idea of successive short holds in propelling the stylets forward is strictly true, but these appear to be contrived by the joint action of the labrum and labium as in the aphis. Close examination of the labium will show that it is equipped with two vice-like processes, one being at the tip of the labium and the other at its base (Fig. 13B).

Between these two processes the tips of the stylets enter the labium. The hinder process is movable and is undoubtedly the tip of the labrum which in the scale enters the labium instead of being superimposed as in the aphis. Fig. 13 shows a short hold on their respective stylets by the labrum and labium of an aphis and a scale. The difference in the method of penetration is that the aphis retracts the long labium into the body and the scale protracts the long stylets out of the body.

The stylets of the aphis are nearly twice the diameter of those of the scale and may be assumed to be twice as stiff. Hence the shortness of the hold would be less and penetration correspondingly much more rapid. The short labrum of the scale, in spite of the pincer-like grip depicted in Fig. 13, has a much weaker hold on a more flexible stylet. This must necessitate a very short hold indeed, with consequently very slow penetration.

These inferences are borne out by sections showing the paths of both aphis and scale stylets through the same tissue. No damage to the cells of the cortex is noticeable in the straight path of the larger aphis stylet which pierces these to reach its objective in the region of the cambium. The stylet of the scale, on the other hand, no sooner enters the cortex than broken-down cells appear in its path, indicating that in its much slower progress the scale has to feed on the cell contents that the tips of the stylets invade (Fig. 14).

The same sections show a marked difference in the control of the stylets by the aphis and the scale. The tips of the aphis stylets make numerous diversions into the surrounding tissue after they are inserted. The length of these diversions corresponds closely to the depth of the buccal cavity in the head into which the stylets can be withdrawn. The mandibular stylets, working independently, are then able to manipulate the tips of the maxillae in new directions. These diversions appear to be entirely absent in the scale, undoubtedly owing to the looped condition of the stylets.

The action of penetration thus becomes more or less automatic in the scale. This was evidenced when a normal adult was turned over, showing that the tips of the stylets had met with some obstruction. The greater part of the stylets had been propelled out of the body without any portion entering the bark. Unlike the aphis the scale has no definite objective and appears satisfied with any tissue of its many host plants. Sections through the annual growth of apple reveal the scale stylets *in situ*. These are shown passing straight through the cortex, being turned aside by a bundle of pericycle fibres, continuing their course through the phloem, crossing the cambium, and invading the immature xylem. They are only prevented from further incursions by the mature fibres of the wood which deflect them into a course parallel with the cambium. Slow penetration and lack of directional control appear to be compensated for by the provision of long stylets.

Within the limited range of these observations, directional control of the stylets is accompanied by a discrimination as to host plants and the tissues attacked. Selective feeding gives rise to specific characters in various galls, by which the species responsible may be identified. A sense of taste is also clearly marked in the higher development of the gustatory organ of the epipharynx in the aphids that form galls. This organ is very reduced in the scale, but the salivary pump above it is correspondingly much enlarged to provide the power needed to force the saliva down the long duct.

In *Adelges cooleyi* Gillette we have a form that brings the aphis and scale into closer relationship. The summer form on Douglas fir is distinctly *Aleyrodid* in character. The first instar has stylets nearly as long as the scale and looped within the body. The labium is longer than that of the scale but the labrum is longer than the labium. The mechanism of the mouth parts is obscure but the rapidity with which the stylets are withdrawn into the body if the insect is disturbed indicates that the labrum plays an important part in penetration.

Acknowledgment

The author wishes to acknowledge the encouragement of Prof. G. J. Spencer of the University of British Columbia and the interest of fellow members of the staff of the Dominion Entomological Laboratory at Vernon, B.C., during the course of the work described.

References

1. BAKER, A. C. The woolly apple aphis. U.S. Dept. Agr., Report 101. 1915.

2. DAVIDSON, J. The structure and biology of *Schizoneura lanigera* Hausmann. Quart. J. Micros. Soc. 58 : 653-701. 1913.

3. DAVIDSON, J. On the mouth-parts and mechanism of suction in *Schizoneura lanigera*. J. Linnean Soc. Zool. 32 : 307-330. 1914.

4. GRISWOLD, G. A study of the oyster-shell scale. Memoir 93. Ithaca. 1925.

5. GROVE, A. J. The anatomy of the head and mouthparts of *Psylla mali*, the apple sucker, with some remarks on the function of the labium. Parasitology, 11 : 456-488. 1919.

6. HERIOT, A. D. Some notes on the oyster shell scale. Proc. Ent. Soc. B.C. No. 28 : 6-13. 1931.

7. IMMS, A. D. General textbook of entomology. E. P. Dutton and company. New York. 1930.

8. IMMS, A. D. Recent advances in entomology. P. Blakiston's Sons and company. Philadelphia. 1931.

9. WEBER, H. Biologie der Hemipteren. Berlin. 1930.

THE ANALYSIS AND COMPOSITION OF THE FLESH OF THE DOMESTIC FOWL[1]

By R. Holcomb[2] and W. A. Maw[3]

Abstract

Determinations have been made of the moisture, fat, ash, and protein content of 34 chickens. The samples consisted of the skin, fat and flesh of the entire carcass. The methods of analysis used were: moisture, drying *in vacuo* at 50° C.; fat, hydrochloric acid digestion; ash, ignition at 700° C.; nitrogen, Gunning modification of the Kjeldahl; protein, by difference. The percentage composition has been found to be:

	Moisture	Fat	Ash	Protein	Nitrogen
Average of 32	65.33	13.99	0.89	19.79	3.298
Standard deviation	4.10	4.99	0.05	1.59	0.507
Maximum	71.81	28.92	0.98	22.33	3.528
Minimum	54.23	6.35	0.77	16.08	2.981

The fat content was found to be inversely proportional to the percentage of moisture, the correlation coefficient between the two being −0.962. A comparison was made of the hydrochloric acid digestion method and the Soxhlet extraction of fat, the former being found to be much superior in reliability of results and in economy of time.

Introduction

In connection with a nutritional study in progress in the Department of Poultry Husbandry of Macdonald College, analyses have been made of representative individuals from lots of chickens in fattening trials under confinement. The aim of the study was to determine the effect of the protein level upon the condition of finish in poultry under forced feeding in preparation for market. A general discussion of the problem and the results of the work up to the present will be given elsewhere. It was thought that in the meantime, a description might be given of the methods of chemical analysis which have been used, together with some observations on the data obtained.

It was decided at the start of the work to analyze the entire edible carcass of each bird rather than to select for examination a certain portion such as a particular muscle tissue. The sample, as received in the laboratory, consisted of the skin and attached fat, together with the muscular flesh (dark and light meat) which had been cleanly removed from the skeletons of those birds that had been chosen from the feeding lots for analysis. The abdominal fat was also included in the sample since the increase in the amount of external fat is apparently closely related to that of the abdominal fat. The constituents to be estimated were the moisture fat, ash and protein.

[1] *Contribution from the Faculty of Agriculture of McGill University, Macdonald College, Macdonald College P.O., Quebec, Canada. Macdonald College Journal Series No. 49.*

[2] *Research Assistant, Department of Chemistry, Macdonald College.*

[3] *Assistant Professor, Department of Poultry Husbandry.*

Crude protein is customarily estimated by multiplying the percentage of nitrogen (Kjeldahl) by a factor. The nitrogen factor for muscular tissue is given as 6.25, for skin and connective tissue as 5.55, and for the nitrogenous bases as small as 3.34. As a consequence of these differences in the nitrogen content of the constituents of meat, and as the relative amounts of the different tissues would be different in the separate samples, it was decided to report the nitrogen content directly and to report the protein by the difference method as first suggested by Atwater (3).

Since meat is composed almost entirely of moisture, fat, ash and protein, the difference between 100 and the sum of the percentages of the three other constituents gives the percentage of crude protein. According to some (2) this figure has often more significance than $N \times 6.25$. With data for the nitrogen content and for the protein by difference of the several birds analyzed, it was possible to compute the nitrogen in the protein fraction and to observe the influence of the nitrogen content of the feed upon this figure.

Description of Methods

Preparation of Sample

The sample as received was first chilled until time was available for work upon it. It was then cut into strips (about 1 in. wide) and put through a meat grinder with the coarse cutter attached. The grinding was repeated ten times, with thorough mixing after each passage through the grinder. As soon as the consistency would allow, the coarse cutter was replaced by one of medium size, usually after the fifth or sixth grinding. Passage of the sample through the grinder only three times was recommended by Wiley (10) and is still to be found in the methods of the A.O.A.C. (1). This may be sufficient for sampling separate tissues, but in the work being described, even after ten grindings individual tissues could be easily recognized. Owing to this inhomogeneity in the sample large weights of material had to be used for the separate determinations.

Moisture

Samples of about 20 to 30 gm. were rapidly weighed (to 0.005 gm.) into salve boxes of tinned metal, $3 \times \frac{3}{4}$ in., with tight fitting covers. About 90% of the moisture was removed at 50° C. *in vacuo* (four days) and the remainder at 105° C. at atmospheric pressure.

Ash

The moisture-free samples from the above determination, added to crucibles in successive small portions, were burned carbon-free at 700° C., the oxidation of the carbonized residues being assisted by small additions of nitric acid.

Fat

A modification of the method given by Buttenberg (4) was devised and found distinctly superior to the Soxhlet both in completeness of extraction and in quality of the extracted fat (see Discussion of Methods). Samples of about 10 to 15 gm. of the original material were rapidly weighed into

150-ml. Erlenmeyer flasks and covered with 25 ml. of concentrated hydrochloric acid. The flasks, loosely covered with small funnels, were left overnight on a boiling water bath. With the aid of hot water, the digested mass, now almost entirely liquid, was transferred in about equal portions to two Mojonnier extraction flasks. After cooling, each tube was extracted with four successive portions of a 1 : 1 mixture of ethyl and petroleum ethers, the Erlenmeyer also being rinsed with the ethers. The extracts were combined in a tared flask, the ether distilled off, and the residue dried at 100° C.

Nitrogen

Samples of about 20 to 30 gm. were digested with 100 ml. of concentrated sulphuric acid and 20 gm. of potassium sulphate-copper sulphate mixture. Five ml. of kerosene was added to prevent foaming. The resulting liquid was made up to 200 ml. with water and aliquots taken for the distillation.

Discussion of Methods

Preparation of Sample

The importance of the lack of homogeneity in the sample is shown by its effect upon the values of the mean deviations in the separate determinations, and the consequent precision of measurement. In Table I the mean deviations in the determination of moisture have been grouped according to size, and averages taken within these groups. The averages for the corresponding deviations in the determination of fat have also been taken. The direct proportionality between the two sets of averages would show that, since the lack of homogeneity is one of the most important sources of error common to both determinations, it is the most influential factor affecting their size.

TABLE I

RELATION BETWEEN DEVIATIONS IN MOISTURE AND FAT

Group		Average of mean deviations	
Range	Number	Moisture	Fat
0.00 to 0.09	12	0.05	0.16
0.10 to 0.19	7	0.13	0.24
0.20 to 0.39	5	0.28	0.30
0.40 to 0.59	4	0.46	0.42
0.60 to 0.79	1	0.64	0.61
0.80 to 0.99	0	—	—
over 1.00	1	1.48	1.72
Left out	4		

Moisture

During the drying at 50° C. the meat retains its flesh color and shrinks to a transparent horn-like mass. The drying is at first more rapid than at higher temperatures, where color changes appear, associated with protein coagulation. The last portions of the moisture are safely and best removed at 100° C.

Fat

Digestion methods for fat in meats were recommended by Konig as early as 1914 (6) and also by Buttenberg (4), but American chemists (1) have adhered to the Soxhlet procedure, in some cases reluctantly (3). Preliminary experiments were made with the latter method both with samples dried at 105° C. in air, and upon those dried at 50° C. *in vacuo*, and with ethyl and

petroleum ethers as extractants. The extractors were heated by electric light bulbs, adjustable as to height, and broken porcelain was used in the flasks to prevent bumping. The fatty residues obtained were dark in color, like molasses, and had a disagreeable burnt odor. They would obviously be unsuited for the determination of physical constants. In contrast, the residues from the digestion method closely resembled hot-water-extracted chicken fat in color and odor and also in the absence of an ultra-violet fluorescence observed in the Soxhlet fats by use of a Callophane filter.

Comparisons of the results obtained by the two methods are given in Table II. It will be seen that, with the exception of the first three samples, the percentage of fat extracted is materially higher by the digestion than by

TABLE II

COMPARISON OF METHODS FOR FAT

Sample No.	Soxhlet			Digestion	
	Dried at	Extractant	% Fat	% Fat	Difference
219	105°	Petrol. ether	13.28	11.33	−1.95
42			9.44	9.26	−1.18
3141			11.93	12.02	0.09
77			5.54	6.35	0.81
3196			8.96	9.80	0.84
4564	105°	Petrol. ether	12.43	13.99	1.56
3119			12.84	14.92	2.08
3140			12.73	16.25	3.52
417			12.65	16.83	4.18
142			14.75	19.58	4.83
1885	50°	Petrol. ether	13.37	16.61	3.24
		Ethyl ether	15.98		0.63

the dry-extraction method. The average difference is 1.64% of the total weight and is equal to 12.0% of the average fat content of the samples. The maximum difference, 4.83% (sample 142), is about 25% of the fat content of the sample or about 32% of that indicated by the Soxhlet method. The average deviation of duplicates was practically the same in the two methods (0.30 and 0.32), showing that both have about the same degree of precision. The digestion method is to be recommended as it requires less time and less fragile apparatus, obviates the necessity of drying the sample and the danger of ignition of the extractant and finally, appears to give more trustworthy results.

Protein

Atwater and others (2, 3, 4, 7) have pointed out that the conventional nitrogen factor, 6.25, does not properly apply to the mixture of proteins of meat. Some advocated the reporting of protein by difference and of nitrogen content as rational independent figures. The factors necessary to convert the nitrogen to protein as obtained by difference varies with the proportions of the various tissues in the sample. Reference to Table III shows that for

TABLE III

THE PERCENTAGE COMPOSITION OF CHICKENS (EDIBLE PORTION MINUS GIBLETS)

Sample	Ration	Moisture	Fat	Protein	Ash	Nitrogen	N in protein, %	Sample
14	II	66.24 ± 0.29	11.07 ± 0.15	21.75 ± 0.45	0.94 —	3.379 ± 0.002	15.54 ± 0.32	14
19	check	68.70 0.14	9.67 0.33	20.68 0.48	0.95 ± 0.02	3.332 0.099	16.11 0.61	19
42	III	70.27 0.08	9.26 0.04	19.56 0.15	0.91 —	3.432 0.010	17.54 0.14	42
66	check	71.14 1.48	10.23 0.72	17.68 2.20	0.95 —	3.256 0.021	18.42 2.29	66
77	V	71.81 0.09	6.35 0.10	20.86 0.20	0.98 0.01	3.521 0.016	16.88 0.18	77
103	check	60.43 ± 0.12	20.36 ± 0.76	18.33 ± 0.98	0.88 ± 0.10	3.141 ± 0.089	17.13 ± 1.04	
142	V	59.82 0.24	19.58 0.64	19.78 0.88	0.82 0.00	3.030 0.019	15.32 0.71	142
144	check	69.25 0.06	10.38 0.25	19.41 0.33	0.96 0.01	3.137 0.051	16.16 0.37	144
172	V	66.23 0.05	10.75 0.24	22.06 0.29	0.96 0.00	3.511 0.006	15.91 0.21	172
219	I	67.88 0.45	11.33 0.32	19.90 0.78	0.89 0.01	3.235 0.016	16.26 0.64	219
349	IV	62.15 ± 0.30	19.59 ± 0.59	17.44 ± 0.89	0.82 ± 0.00	—		349
358	III	60.56 0.03	18.94 0.55	19.69 0.59	0.81 0.01	—		358
417	IV	62.15 0.20	16.83 0.06	20.16 0.27	0.86 0.01	3.321 ± 0.006	16.47 ± 0.22	417
789	check	67.65 0.06	10.80 0.18	20.61 0.24	0.94 0.00	3.414 0.038	16.56 0.24	789
1051	check	65.10 0.09	13.13 0.33	20.89 0.42	0.88 0.00	3.201 0.042	15.32 0.31	1051
1085	IV	63.99 ± 0.01	13.91 ± 0.10	21.19 ± 0.12	0.91 ± 0.01	3.625 ± 0.059	17.11 ± 0.29	1085
1088	V	64.47 0.05	16.31 0.05	18.36 0.12	0.86 0.02	—		1088
1142	III	70.29 0.16	6.53 0.07	22.33 0.24	0.85 —	—		1142
1225	I	63.05 0.10	11.17 0.05	24.88 0.16	0.90 0.01	—		1225
1269	II	62.78 0.11	17.16 0.12	19.22 0.24	0.84 0.01	—		1269
1354	V	64.12 ± 0.01	15.14 ± 0.10	19.86 ± 0.12	0.88 ± 0.01	—		1354
1364	II	63.22 0.18	11.85 0.25	24.07 0.45	0.86 0.02	—		1364
1448	III	54.23 0.37	28.92 0.08	16.08 0.46	0.77 0.01	—		1448
1864	I	64.84 1.57	14.54 0.17	19.75 1.77	0.87 0.03	3.220 ± 0.031	16.30 ± 1.24	1864
1885	II	63.59 0.64	16.61 0.61	18.92 1.26	0.88 0.01	3.112 0.003	16.50 1.09	1885
2818	I	57.65 ± 0.12	24.13 ± 0.67	17.45 ± 0.79	0.77 ± 0.00	—		2818
3108	check	71.59 0.11	8.45 0.12	19.02 0.25	0.94 0.02	2.981 ± 0.065	15.67 ± 0.39	3108
3119	III	64.32 0.09	14.92 0.37	19.89 0.37	0.87 0.01	3.261 0.037	16.40 0.35	3119
3140	IV	61.88 0.03	16.25 0.07	20.93 0.11	0.94 0.01	3.528 0.064	16.85 0.32	3140
3141	II	66.65 0.45	12.02 0.79	20.43 1.45	0.90 0.01	3.122 0.036	15.28 0.92	3141
3148	IV	67.58 ± 0.51	10.85 ± 0.38	20.68 ± 0.89	0.89 ± 0.00	3.353 ± 0.018	16.21 ± 0.70	3148
3176	I	68.54 0.42	9.87 0.20	20.71 0.63	0.88 0.01	3.356 0.039	16.20 0.56	3176
3196	I	68.53 0.03	9.80 0.60	20.73 0.64	0.94 0.01	3.392 0.023	16.36 0.54	3196
4564	II	66.15 0.01	13.97 0.17	18.94 0.20	0.94 0.02	3.282 0.032	17.35 0.25	4564
Average (32)		65.33 ± 4.10	13.99 ± 4.99	19.79 ± 1.59	0.89 ± 0.05	3.298 ± 0.51	16.41 ± 0.70	

the samples of poultry meat here reported, it would vary from 5.43 (sample 66) to 6.53 (sample 142) and would average 6.094, corresponding to a nitrogen content of 16.42% in the protein.

The objection that, by estimating protein by difference, one is imposing upon this constituent the accumulated errors of the other three—fat, ash and moisture—is not, in our opinion, a very serious one. In the results reported in Table III the mean deviation of the protein by difference is 0.57 or 2.87% of the average protein content while that for nitrogen is 0.034 or 1.05% of the average nitrogen content.

Results

Composition

The composition of the 34 chickens analyzed is set out in Table III. The five rations referred to in the second column are described in Table IV,

TABLE IV

PERCENTAGE COMPOSITION OF THE RATIONS

Ration No.	Moisture	Fat	Fibre	N × 6.25	Ash	N-free extract
I	10.56	5.24	2.78	14.18	2.53	64.71
II	10.33	4.78	2.60	16.42	3.19	62.68
III	10.17	5.34	2.48	18.09	3.51	60.41
IV	10.36	5.40	2.27	19.64	4.60	57.73
V	10.18	5.72	2.13	22.69	5.79	53.49

although the discussion of the effect of these rations upon the composition will be given elsewhere. The percentage by weight of each constituent is supplemented by a figure representing the average deviations of the duplicate determinations from their mean. In studying the data with reference to the influence of the feeding upon the composition, as will be described elsewhere, this figure may serve as a rough measure of the value of the mean percentage. The mean deviations for the protein by difference are the sums of the deviations of the three other constituents. The figure given as the mean deviation for the percentage of nitrogen in the protein by difference has been calculated from the relation,

$$d_{NP} = \sqrt{\left(\frac{100d_N}{P}\right)^2 + \left(\frac{100d_P N}{P^2}\right)^2}$$

where N and P are the percentages of nitrogen and protein respectively, d_N and d_P are the corresponding deviations for these two percentages, and d_{NP} is the "precision measure" of the percentage of nitrogen in the protein.*

At the foot of the table is given the average composition of 32 birds (Nos. 1225 and 1364 have been excluded for reasons to be given below). The standard deviations of the groups from these averages are also given as a measure of the range of variation of each constituent.

* *The following texts have been used as guides in the statistical examination of the analytical data: Goodwin's Precision Measurements and Graphical Methods, New York, 1920; Tippett's Methods of Statistics, London, 1930; and Bowley's Elements of Statistics, 5th ed., London, 1926*

Although these chickens had been selected for the feeding experiment because of their uniformity, were of about the same age, and had been fed almost similarly in the trials, there is a wide range in their composition. The percentage of moisture ranges from 54.23 to 71.81 with an average of 65.33. The percentage of fat shows the same wide variation, ranging from 6.35 to 28.92 with an average of 13.99. The variation in the percentages of ash, protein, and nitrogen is much less, as shown by the relatively small standard deviations.

There appears to be a remarkable individuality in the composition of animals. This has been recognized (8) as one of the major difficulties in the study of animal response in nutritional investigations. As has been noted, even the percentage of nitrogen in the protein fraction, a ratio which one would imagine to be fairly constant for any animal species, shows a wide variation.

Relation between Constituents

During the progress of the analyses, it was noticed that there was an inverse proportion between the percentages of fat and moisture. A scatter diagram of this relation is shown in Fig. 1, each point representing the corresponding fat and moisture content of one bird. The linear regression equation, $F = -1.170\,M + 90.48$, is drawn in to show the regular distribution over the range. Since the composition of birds No. 1225 and No. 1364 (marked by squares) appeared irregular, it was suspected that either the samples as received, or the birds themselves were abnormal in composition, or that some error had crept into the analysis. Two additional birds from the same feeding lots were analyzed to replace the two of doubtful composition.

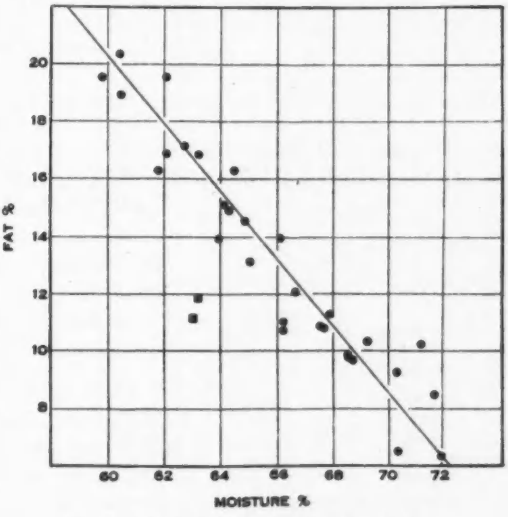


FIG. 1. *Relation between fat and moisture.*

To measure the degree of association between the two constituents for the 32 pairs, the correlation coefficient was computed and found to be -0.962. This figure is sufficiently close to -1.0 to be considered as an almost perfect association. The two regression equations were found to be,

$$F = -1.170\,M + 90.48,$$

and

$$M = -0.791\,F + 76.40,$$

the former giving the best value for the percentage fat calculated from any moisture content, and the latter the best value of the percentage of moisture calculated from any percentage of fat. Their value is shown as a guide in judging the worth of the results of the analyses of birds No. 1225 and No. 1364, the deviations in these cases being over four times the average deviation.

From a biological viewpoint this relation between fat and moisture is especially interesting as the fat in birds, for the most part, is laid down under the skin and within the abdomen, while the moisture is fairly evenly distributed through the muscular and connective tissue.

This relation between fat and moisture was pointed out by Wait (9) who concluded from his results that, "the fluctuations in the amount of fat follow almost exactly the fluctuations in the amount of water, - - - - -. This relation of fat and water holds true for nearly all kinds of flesh which have been analyzed."

A notable example of this relation is to be found in the work of Haecker (5), although it was neither noted nor discussed by him. From his data given for the percentage composition of steers, the correlation coefficient for the percentages of moisture and fat in the carcass is found to be -0.997, the regression equations corresponding to those given above, being,

$$F = -1.18\,M + 88.71,$$

and

$$M = -0.83\,F + 74.71.$$

The small differences in the constants in these two sets of equations is more than likely due to the natural differences in the composition of steers and chickens, although these unrelated animal species show the same relation between their fat and moisture contents. When additional analytical data are at hand, it will be interesting to see if this relationship is general in the animal kingdom.

TABLE V

RELATION BETWEEN MOISTURE AND ASH

Per cent ash	Average percentage moisture
0.77	55.94
0.81	60.56
0.82	60.98
0.84	62.78
0.85	70.29
0.86	63.31
0.87	64.85
0.88	64.36
0.89	67.73
0.90	66.65
0.91	67.13
0.94	68.03
0.95	68.92
0.96	67.74
0.98	71.81

There are no very definite relations between any of the other constituents. The correlation coefficient for the association of moisture and protein is $+0.426$, which, however, is too low to be considered significant from a practical point of view. The percentages of ash and moisture are only roughly proportional. The percentages of moisture corresponding to the different percentages of ash have been averaged and are shown in Table V. As the moisture content of the meat increases so also does the ash. The increase is far from regular but with the exclusion of three pairs (0.85, 0.89 and 0.96) a general parallelism is seen.

Acknowledgment

We take pleasure in acknowledging our indebtedness to Dr. J. F. Snell for many suggestions and for his continued interest in the work, and also to Dr. W. W. Stewart and Dr. Darol K. Froman for their kind assistance with portions of the mathematical treatment.

References

1. Association of Official Agricultural Chemists. Methods of Analysis, 3rd ed., Section 27, page 277. Washington, 1930.
2. Atwater, W. O. and Bryant, A. P. The chemical composition of American food materials. U.S. Dept. Agr., Exp. Sta. Bull. 28. 1899.
3. Atwater, W. O. and Woods, C. D. The composition of food materials. Storrs Agr. Exp. Sta. 4th Annual Report, page 46, 1891.
4. Buttenberg, P. Meat and meat preparations. Abderhalden's Handbuch der biologischen Arbeits-methoden, vol. 8, section 4, pt. 1, page 679. 1923.
5. Haecker, T. L. Investigations in beef production. Minn. Agr. Exp. Sta. Bull. 193. 1920.
6. Konig, J. Chemie der menschlichen Nahrungs- und Genussmittel, vol. 3, pt. 2, 4th ed. Berlin, 1914.
7. Richardson, W. D. and Scherubel, E. The deterioration and commercial preservation of flesh foods. J. Am. Chem. Soc. 30 : 1515. 1908.
8. Sheets, E. W. Meat research in the United States. Sci. Monthly, 38 : 50. 1934.
9. Wait, C. E. Nutritional investigations at the University of Tennessee. U.S. Dept. Agr., Exp. Sta. Bull. 53. 1898.
10. Wiley, H. W. A preliminary study of the effects of cold storage on eggs, quail and chickens. U.S. Dept. Agr. Bur. of Chem. Bull. 115. 1908.

INFLUENCE OF CYSTEINE ON THE PRODUCTION OF HAEMOTOXIN OF *CL. WELCHII*[1]

By J. H. Orr[2] and G. B. Reed[3]

Abstract

It has been shown that the addition of 0.1% or more of cysteine to Robertson's chopped meat media completely inhibits the production of haemotoxin by *Cl. welchii* and that H_2S has a similar effect. The effect is shown to be related to the metabolism of the organisms and not to a direct reaction with the formed toxin. The addition of these concentrations of cysteine was shown to have little effect upon the oxidation-reduction potential of sterile media but a marked effect upon the oxidation-reduction potential of cultures during the most active growth period. Beef muscle media low in cystine were shown to give a good yield and fish muscle relatively high in cystine was shown to give a poor yield of haemotoxin.

During the course of an investigation of the haemotoxin of *Cl. welchii*, Reed, Orr and Burleigh (6), Orr, Campbell and Reed (4), we have experienced much difficulty with variation in yield of haemotoxin from the same cultures.

The problem of the variability of the organisms has recently been discussed by Orr, Josephson, Baker and Reed (5). In the later work on toxin production the only cultures used have been those which have been so stabilized by selection that they fail to show variation as determined by colony structure and by agglutination reactions. But notwithstanding apparent uniformity of type in successive cultural generations, wide variation in haemotoxin yield has frequently been observed. It seemed apparent, therefore, that the irregularity was dependent upon lack of uniformity in the culture media or in some other environmental factors.

The amino acid content of the medium was shown by Davis and Ferry (2) and some others to have a conspicuous influence upon the yield of diphtheria toxin and recently Walbum and Reymann (9) have shown that the increase in amino nitrogen during the course of development of a culture of *Cl. welchii* runs parallel, up to a maximum amount, to the production of killing toxin. We therefore undertook an investigation of the influence of the amino acid content of the culture media upon the yield of haemotoxin.

Influence of Amino Acids on Haemotoxin Production

A series of flasks was arranged, each containing 100-cc. portions of Robertson's chopped-beef medium with 0.1% glucose. To each flask was added one of the following amino acids to a concentration of 0.1%: alanine, cysteine, cystine, tyrosine, histidine, tryptophane and glycine together with creatin and creatinine in the same concentration. The flasks were boiled, rapidly cooled, and inoculated with an S type toxin-producing strain of *Cl. welchii*,

[1] *Contribution from the Department of Bacteriology, Queen's University, Kingston, Ontario, Canada.*

[2] *Assistant Professor of Bacteriology, Queen's University.*
[3] *Professor of Bacteriology, Queen's University.*

sealed with vaseline and incubated at 37° C. for 21–22 hr. After filtration the filtrates were titrated against washed rabbit red cells. This titration was carried out immediately with a minimum exposure to air, to reduce the possibility of oxidation of the toxin as such a reaction was shown by Neill (3) and ourselves (7) to be an important factor in the activity of haemotoxins.

Haemotoxin titrations were made by setting up serial dilutions of the toxic filtrates in freshly boiled saline to which washed rabbit red cells to 2% were added. After three hours' incubation, with frequent shaking, in a 37° C. water bath the maximum dilution of the toxin which caused haemolysis of half the cells was determined by matching the centrifuged reaction tubes against a color standard.

The results shown in Table I indicate clearly that two of the amino acids, cystine and cysteine, have a profound inhibitory effect upon the haemotoxin

TABLE I

TITRATION OF *Cl. welchii* HAEMOTOXINS PREPARED FROM MEDIA CONTAINING DIFFERENT AMINO ACIDS, CREATIN, AND CREATININE

Amino acid, 0.1%	Toxin concentration												
	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240	1/20480	1/40960	Control
Cysteine	+	−	−	−	−	−	−	−	−	−	−	−	−
Cystine	+	+	−	−	−	−	−	−	−	−	−	−	−
Alanine	+	+	+	+	+	+	+	+	+	±	−	−	−
Tyrosine	+	+	+	+	+	+	+	+	±	±	−	−	−
Histidine	+	+	+	+	+	+	+	+	+	+	−	−	−
Glycine	+	+	+	+	+	+	+	+	+	+	±	−	−
Tryptophane	+	+	+	+	+	+	+	±	−	−	−	−	−
Creatin	+	+	+	+	+	+	+	+	+	±	−	−	−
Creatinine	+	+	+	+	+	+	+	+	+	+	±	−	−
No addition	+	+	+	+	+	+	+	+	+	+	−	−	−

production by this organism and that the other amino acids tested are without effect in the concentration tested. The experiment has been repeated many times—the addition of 0.1% of cysteine to the chopped meat medium has never failed to inhibit haemotoxin formation. The fact that alanine, which has the same structure as cysteine except for the absence of the SH group, shows no inhibitory effect, suggests that the inhibition is due to the sulphur or the SH group. The difference in the solubility of cysteine and cystine probably accounts for the greater inhibitory activity of the former.

In order to determine the concentration of cysteine necessary to bring about inhibition, a series of flasks of chopped meat media containing decreasing concentrations of the cysteine, ranging from the 0.2 to 0.001% were inoculated with the same culture as was used in the last experiment. The results shown in Table II indicate that concentrations less than 0.1% have very little effect.

TABLE II

TITRATION OF *Cl. welchii* HAEMOTOXIN PREPARED FROM MEDIA CONTAINING INCREASING CONCENTRATIONS OF CYSTEINE

Cysteine, %	Toxin concentration											
	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240	Cont.
.2	+	−	−	−	−	−	−	−	−	−	−	−
.1	+	−	−	−	−	−	−	−	−	−	−	−
.05	+	+	+	+	+	+	+	+	±		−	−
.01	+	+	+	+	+	+	+	+	±	−	−	−
.05	+	+	+	+	+	+	+	+	+	+	±	−
.001	+	+	+	+	+	+	+	+	+	+	±	−

The ability of sulphur in other forms to effect the haemotoxin production of this organism was also examined. Hydrogen sulphide was bubbled through sterile distilled water to the saturation point. The solution was then added to the medium to a concentration of 2%. The medium was sufficiently buffered that this addition of hydrogen sulphide did not alter the pH. As indicated in Table III, *Cl. welchii*, though growing luxuriantly in this medium,

TABLE III

TITRATION OF *Cl. welchii* HAEMOTOXIN PREPARED FROM MEDIA WITH AND WITHOUT ADDED HYDROGEN SULPHIDE

—	Toxin concentration									
	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	Control
Hydrogen sulphide	−	−	−	−	−	−	−	−	−	−
Control	+	+	+	+	+	+	+	+	−	−

failed to produce sufficient haemotoxin to show a reaction in a dilution of 1 in 20 while control toxin prepared in the same batch of medium lacking the hydrogen sulphide gave haemolysis in a dilution of 1 in 500.

In order to ascertain whether the sulphur compounds influenced the metabolism of the organisms or reacted with the haemotoxin, the following experiment was arranged. A sample of toxin prepared in the usual way was divided into two lots. To one lot saturated hydrogen sulphide solution was added to make 2% of saturation as used in the previous experiment, while the other portion was left untreated to serve as a control. The two samples were then sealed with vaseline and allowed to stand for several days. On titration the two toxins gave the same reaction as shown in Table IV. This appears to indicate quite definitely that sulphur or sulphur compounds affect haemotoxin production by *Cl. welchii* through alteration in the metabolism of the organism rather than by acting on the haemotoxin directly.

TABLE IV

TITRATION OF *Cl. welchii* TOXIN WITHOUT ADDED HYDROGEN SULPHIDE AND AFTER STANDING SEVERAL DAYS IN CONTACT WITH HYDROGEN SULPHIDE

—	Toxin concentration										
	1/5	1/10	1/20	1/40	1/100	1/200	1/400	1/800	1/1600	1/3200	Cont.
H_2S-treated toxin	+	+	+	+	+	+	+	+	±	−	−
Normal toxin, no H_2S	+	+	+	+	+	+	+	+	+	−	−

Oxidation-reduction Potential

It was anticipated that the metabolic influence might result from alteration in the oxidation-reduction potential produced especially by the cysteine. Several determinations have been made by the conventional methods. The particular arrangement of the apparatus was as described earlier by Boyd and Reed (1). The culture vessels consisted of 10 × 1 in. Pyrex test tubes containing 35-cc. amounts of the Robertson's meat medium with or without cysteine. The reaction in each case was adjusted to pH 7.2. These were autoclaved with bright platinum electrodes in place. As soon as sufficiently cooled, potassium chloride-agar bridges were inserted and the sterile tubes sealed with a thick layer of vaseline. These were held for 24 hr. at 37° C. and the initial *sterile* potential reading taken at the end of this period (1). Inoculations were made through the vaseline seal with a fine Pasteur pipette from a young culture in the same medium, the cultures maintained at 37° C., and potential readings made at short intervals for 18 to 24 hr. The results have been calculated in terms of the H electrode as zero.

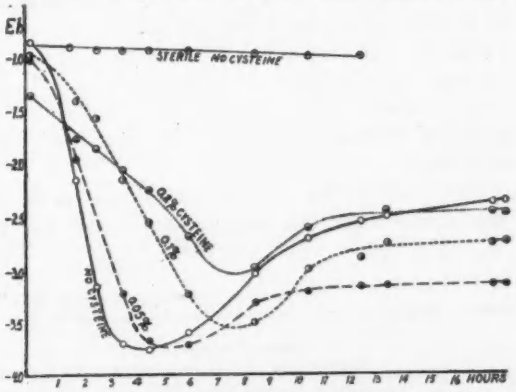


FIG. 1. *Curves indicating change in the oxidation-reduction potential of cultures of Cl. welchii in Robertson's meat media containing various concentrations of cysteine. Ordinates, Eh; abscissa, time in hours.*

A characteristic result is shown in the curves (Fig. 1) drawn from the results of an experiment in which three concentrations of cysteine, 0.05, 0.1 and 0.2% were used, together with a control without added cysteine and with a sterile control. The cysteine and control tubes were filled from one flask of Robertson's chopped meat in such a way that each received the same amount of both the solid meat and the fluid portion of the medium. It is apparent that the addition of cysteine up to 0.1% produced no appreciable change and 0.2% produced a barely significant change in potential in the

sterile medium. This condition doubtless results from an efficient poising effect of some constituent of the medium. On the other hand the poising action of the cysteine is very apparent, particularly during the first six to eight hours' growth of the culture. The curves (Fig. 1) indicate that in the cultures without cysteine there is a precipitous drop in oxidation-reduction potential which begins in slightly less than an hour after inoculation, followed by a gradual rise to an approximate equilibrium at 13–15 hr. Cultures containing 0.05% cysteine show a similar, although slightly more delayed, drop, followed by a more gradual rise. The 0.1 and the 0.2% cysteine cause a much greater delay in the initial fall and definitely restrict the extent of the fall. This appears unrelated to the rate of growth of the organisms. Although no quantitative determinations were made, the rate of pH change in the controls and in the cysteine-containing media was approximately the same and the first evolution of gas occurred in all the tubes at two and a half to three hours after inoculation. Since the pH change was approximately the same in all the tubes, from 7.2 to 6.2 six to eight hours after inoculation, no correction for this change has been made in the curves. Part, but not all, of the positive drift six to eight hours after the inoculation is the result of the pH change.

It appears not unreasonable to conclude that the marked differences in the oxidation-reduction of the medium during the most active period of the growth of the organisms may have a profound influence on the metabolism, and result in the observed differences in haemotoxin production.

Cystine Content of Various Media

Sullivan and Hess (8), employing several different methods for the quantitative estimation of cystine have demonstrated the existence of a very appreciable difference in the amount of this amino acid contained in various kinds of meat and fish. As shown in Table V, while there is some slight variation in the amount of cystine shown in the materials tested by the different methods, all are in agreement so far as showing that fish muscle contains in the neighborhood of twice the amount of cystine contained in beef muscle.

A series of media were made up similar to Robertson's chopped meat, using sirloin and round beef steak, salmon and halibut muscle. The media were

TABLE V

CYSTINE CONTENT OF FRESH MEAT AND FISH

—	Cystine on moist weight, %				Cystine on dry weight, %			
	Sullivan method	Okuda method	Folin Marenzi method	Average	Sullivan method	Okuda method	Folin Marenzi method	Average
Round steak	.132	.130	.174	.145	.522	.516	.693	.577
Sirloin steak	.118	.117	.194	.143	.194	.480	.474	.382
Haddock	.231	.231	.387	.283	1.16	1.16	1.94	1.42
Halibut	.238	.231	.408	.292	1.13	1.09	1.94	1.38
Salmon	.236	.243	.418	.299	.85	.88	1.50	1.07

TABLE VI

TITRATION OF *Cl. welchii* TOXIN PREPARED FROM MEAT AND FISH MEDIA

—	Toxin concentration										
	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{400}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{4000}$	$\frac{1}{8000}$	$\frac{1}{16000}$	$\frac{1}{32000}$	Cont.
Sirloin steak	+	+	+	+	+	+	±	±	−	−	−
Round steak	+	+	+	+	+	+	±	−	−	−	−
Halibut	+	+	+	±	±	−	−	−	−	−	−
Salmon	+	+	+	±	±	−	−	−	−	−	−

all prepared in the same manner, all adjusted to pH 7.2 and dispensed to small flasks with care that all the flasks received the same proportion of solid and fluid portions of the media. The percentage content of cystine to be expected in the several media from Sullivan and Hess' results is shown in the last column of Table II. It is not apparent, however, what proportion is present as free cysteine or combined in proteins. The media were all inoculated with the same toxin producing strain of *Cl. welchii* and filtrates recovered from all after 22 hours' incubation. The results of haemotoxin titration are shown in Table III. It is apparent that the beef muscle media with a low cysteine content give a high yield of toxin and the fish muscle media with a much higher content of cystine give a very much lower yield. It is of course probable that other constituents of the two media are also concerned.

Conclusion

1. It has been shown that the addition of 0.1% or more of cysteine to Robertson's chopped meat media completely inhibits the production of haemotoxin by *Cl. welchii*, and that hydrogen sulphide has a similar effect.

2. The effect is shown to be related to the metabolism of the organisms and not to a direct reaction with the formed toxin.

3. The addition of these concentrations of cysteine were shown to have little effect upon the oxidation-reduction potential of sterile media but a marked effect upon the oxidation-reduction potential of cultures during the most active growth period.

4. Beef muscle media low in cystine was shown to give a good yield, and fish muscle relatively high in cystine was shown to give a poor yield of haemotoxin.

References

1. BOYD, E. M. and REED, G. B. Can. J. Research, 4 : 605-613. 1931.
2. DAVIS, L. and FERRY, N. S. J. Bact. 4 : 217-241. 1919.
3. NEILL, J. M. J. Exptl. Med. 44 : 199-213, 215-226, 227-240. 1926.
4. ORR, J. H., CAMPBELL, W. A. and REED, G. B. Can. J. Research, 2 : 91-100. 1930.
5. ORR, J. H., JOSEPHSON, J. E., BAKER, M. C. and REED, G. B. Can. J. Research, 9 : 350-359. 1933.
6. REED, G. B., ORR, J. H. and BURLEIGH, C. H. Can. Med. Assoc. J. 16 : 525-531. 1926.
7. REED, G. B., ORR, J. H. and CAMPBELL, W. A. J. Infectious Diseases, 41 : 434-438. 1927.
8. SULLIVAN, M. X. and HESS, W. C. Supplement No. 49 to the U.S. Pub. Health Repts. 1931.
9. WALBUM, L. E. and REYMANN, C. G. J. Path. Bact. 36 : 469-483. 1933.

DERIVATIVES OF SUBSTITUTED SUCCINIC ACIDS

I. THE ACTION OF ALKALINE SODIUM HYPOBROMITE ON 1,2-DIPHENYLSUCCINAMIDE AND 3,4-DIPHENYLSUCCINIMIDE[1]

By John A. McRae[2] and Alvin S. Townshend[3]

Abstract

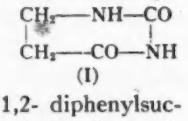
The application of the Hofmann reaction for the preparation of amines from amides to 1:2-diphenylsuccinamide has led to none of the expected substances, but to diphenylacetic acid as the principal product of the reaction. The isolation of this substance indicates that at some stage a rearrangement has occurred. The mechanism of the rearrangement is obscure but oxidation to a 1,2-dihydroxydiamide may be the first stage. This is supported by the conversion of diphenylsuccinimide into diphenylmaleic anhydride by alkaline sodium hypobromite.

Various derivatives of diphenylsuccinic acid, which were obtained in unsuccessful attempts to prepare in quantity other 1:2-diarylsuccinamides, are described.

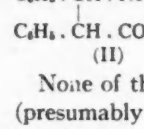
Introduction

The preparation of substituted succinic acids through addition of hydrogen cyanide to substances of the types $RCH : C(CN)CO_2H$ and $RCH : C(C_6H_5)CN$ was studied some years ago by Lapworth and McRae (8). It has seemed desirable to study in several directions the behavior of the acids thus obtained and that of related compounds.

Through earlier work (9) on the action of alkaline sodium hypobromite on phenyl- and piperonyl- succinimides, in which it was found that hydrolysis to the corresponding succinic acids occurred exclusively, the writers' attention was turned first of all to the action of this reagent on 1,2-diphenylsuccinamide which had been obtained by the hydrolysis of 1,2-diphenylsuccinonitrile (8). The only amides of the succinic acid series whose behavior towards sodium hypobromite has been studied previously are succinamide and methylsuccinamide. Weidel and Roithner (11) observed that these amides behaved similarly to phthalamide (7) giving the cyclic ureides, dihydrouracil (I) and methyldihydrouracil, respectively. Accordingly it was expected that 1,2- diphenylsuccinamide would yield diphenyldihydrouracil (II), or alternatively 1,2-diphenylethylene diamine, $\alpha\beta$-diphenyl-β-aminopropionic acid or some other of the various types of substances that sometime arise in the application of the Hofmann reaction.



(I)



(II)

None of the expected substances was found when 1,2-diphenylsuccinamide (presumably the meso- form) was subjected to the action of alkaline sodium hypobromite as employed in the Hofmann reaction. Cold alkaline sodium hypobromite slowly dissolves this amide and when the solution is heated to 75–80° C., after first adding the appropriate quantity of sodium hydroxide,

[1] *Manuscript received July 16, 1934.*

Contribution from the Chemical Laboratories, Queen's University, Kingston, Ontario, with financial assistance from the National Research Council of Canada.

[2] *Professor of Chemistry, Queen's University.*

[3] *Chemist, Guaranty Silk and Dyeing Co., St. Catharines; holder of a bursary, 1924-1925, under the National Research Council of Canada.*

a small amount of alkali-insoluble material is produced, identified as principally diphenylsuccinamide; benzaldehyde and ammonia are formed to a certain extent and on acidifying the filtrate, diphenylacetic acid is precipitated as the principal product of the reaction. It is accompanied by traces of other substances from which it is freed with some difficulty. The yield of diphenylacetic acid varies considerably but in some experiments it has been as high as 90%. Similar results have been obtained using alkaline sodium hypochlorite.

The conversion of diphenylsuccinamide into diphenylacetic acid adds another example to the already numerous instances of rearrangements which various derivatives of 1,2-diphenylethane undergo, although as far as the writers have been able to ascertain alkaline sodium hypobromite has not been found previously to cause such changes. The rearrangement observed is not to be ascribed to the action of hot caustic soda on the diamide, for at the concentration of alkali and at the temperature employed in this attempted Hofmann reaction, caustic soda is without appreciable effect on diphenylsuccinamide. In the second place, although a residue of this amide remains, yet the original diphenylsuccinamide is dissolved by cold sodium hypobromite solution. The authors hope to report shortly on the nature of the changes at this stage. In view of the fact that Feist and Arnstein (5) observed that 1,2-diphenylethylene diamine, when treated with nitrous acid, gave hydrobenzoin and diphenylacetaldehyde, the possibility that this diamine had been formed during the reaction and then either had been oxidized to hydrobenzoin or had undergone rearrangement was investigated. Diphenylethylene diamine was prepared according to Feist (4) by reduction of α-benzildioxime. In this reduction not only was there isolated the racemic form (m.p. 90–91° C.) of the diamine that Feist had obtained, but also the meso- form (m.p. 120° C.) obtained by Grossmann (6) from amarin. Using the same conditions of alkalinity and temperature as were used with diphenylsuccinamide both of these forms with alkaline sodium hypobromite gave benzaldehyde and ammonia, but we were unable to isolate any diphenylacetic acid.

Another possibility is that the hypobromite oxidizes diphenylsuccinamide to $\alpha\alpha'$-dihydroxy- $\alpha\alpha'$-diphenylsuccinamide (III) and the observed changes arise from the further transformations of this substance. Evidence will be presented in a forthcoming paper to support this view, but the probability of such an attack is strengthened by the fact that when 3,4-diphenylsuccinimide was subjected to the action of sodium hypobromite, under the conditions of the Hofmann reaction used for converting phthalimide into anthranilic acid, the expected $\alpha\beta$-diphenyl-β-aminopropionic acid was not isolated, but instead diphenylsuccinamic acid, $C_6H_5CH\,.\,(CONH_2)\,CH(C_6H_5)CO_2H$, and diphenylmaleic anhydride were obtained. The latter was formed most probably by loss of water from α-hydroxy- $\alpha\alpha'$-diphenylsuccinamide, formed by oxidation and hydrolysis of the imide. When diphenylsuccinamic acid was treated with the alkaline hypobromite solution a mixture of acidic substances was produced from which only benzoic acid was isolated.

$$\begin{array}{l} C_6H_5\,.\,C(OH)\,.\,CONH_2 \\ | \\ C_6H_5\,.\,C(OH)\,.\,CONH_2 \end{array}$$
(III)

Attempts were made to ascertain whether the arrangement observed with diphenylsuccinamide and sodium hypobromite was characteristic of 1,2-diarylsuccinamides. For this purpose *pp'*-dinitrodiphenylsuccinamide was prepared by the nitration of diphenylsuccinamide. Its orientation follows from its hydrolysis to the corresponding acid identical with the *pp'*-dinitrodiphenylsuccinic acid described by Reimer (10). Unsuccessful attempts were made to prepare in quantity *p*-methoxydiphenylsuccinamide and *o*-chlorodiphenylsuccinamide. As has also been shown by Brand and Loehr (2) anisylidenebenzyl cyanide readily unites with hydrogen cyanide but the resulting dinitrile, $CH_3OC_6H_4CH(CN)CH(C_6H_5)CN$, could not be hydrolyzed to the diamide. Using a concentration of sulphuric acid appropriate for the hydrolysis, sulphonation occurred. Further heating of the sulphonated product with moderately concentrated sulphuric acid gave *p*-methoxydiphenylsuccinic acid which was prepared by Brand and Loehr by direct hydrolysis. *o*-Chlorophenylcinnamonitrile with hydrogen cyanide unexpectedly gave a tarry product from which the writers were unable to isolate any of the expected dinitrile, but hydrolysis of the material yielded small amounts of *o*-chlorodiphenylsuccinamide and *o*-chlorodiphenylsuccinic acid.

The procedure used with diphenylsuccinamide was applied to its dinitroderivative, but the only substance isolated in amount sufficient for identification was the half-amide of *pp'*-dinitrodiphenylsuccinic acid.

Later work (3) however has shown that under the influence of alkaline sodium hypobromite, 1,2-*p*-tolylphenylsuccinamide gives *p*-tolylphenylacetic acid, and 1,2-*p*-chlorodiphenylsuccinamide gives *p*-chlorodiphenylacetic acid. It may, therefore, be considered that the observed rearrangement is general with 1,2-diarylsuccinamides.

Experimental

Action of Sodium Hypobromite on Diphenylsuccinamide

s-Diphenylsuccinamide was made according to the directions of Lapworth and McRae (8). In general the product obtained from the hydrolysis of diphenylsuccinonitrile was used directly after a thorough digestion with cold sodium hydroxide solution. Using a recrystallized sample of the amide, the same results were obtained as with material which had not been recrystallized.

The hypobromite solution was made by dropping slowly, with efficient stirring, 32 gm. of bromine into a solution of 40 gm. of sodium hydroxide in 200 cc. of water cooled to −10° C. The diphenylsuccinamide (26.8 gm.), made into a thin paste with a little water, was added slowly, the temperature being maintained below 0° C. The agitation was continued for one-half hour. All but a very small residue dissolved. When the temperature had risen to 0° C., 24 gm. of sodium hydroxide was added with stirring and the mixture heated on the water bath to 75–80° C. for three hours. During the heating some ammonia and benzaldehyde were formed and a further precipitate settled out. After dilution this was collected and recrystallized from glacial acetic acid; m.p. 310° C. It did not depress the melting point of diphenylsuccinamide.

Analysis confirmed the identity of the substance as diphenylsuccinamide. Calcd. for $C_{16}H_{16}O_2N_2$: N, 10.45%. Found: N, 10.90%. The substance was identified further by hydrolysis to diphenylsuccinic acid; m.p. 229° C. It is accompanied apparently at times by a small amount of a second substance, comparatively readily soluble in alcohol. On recrystallization from alcohol it melted at 308° C. and depressed the melting point of the substance with which it was associated to 254° C.

The filtrate was extracted with ether but only traces of bases or other ether-soluble substances were obtained. On acidification with hydrochloric acid a yellowish semi-oily precipitate was formed from which carbon dioxide seemed to be evolved gradually. When the precipitate had hardened it was collected, dissolved in sodium hydroxide and the alkaline solution boiled with charcoal. On acidification fairly pure diphenylacetic acid was obtained; yield, 17 gm. It was recrystallized several times from hot water; m.p. 146° C. The equivalent calculated for $C_{14}H_{12}O_2$ is 212; found, 212.5. The substance was compared, and found to be identical, with a sample of diphenylacetic acid, purchased from British Drug Houses, which on recrystallization melted at 146° C. Comparison was made also with a specimen of the acid made by reducing benzilic acid.

A portion of the diphenylacetic acid thus obtained from diphenylsuccinamide was oxidized with chromic acid in glacial acetic acid to benzophenone (m.p. 46° C.). Other portions were converted into the ethyl ester (m.p. 58° C.) and into the anilide (m.p. 180° C.). In each case the preparations were compared with the same derivatives made from authentic diphenylacetic acid, and identified further by analysis.

In an experiment in which the reaction mixture was heated for but one-half hour at 80° C. the yield of diphenylacetic acid fell to 50%. Using one molecular proportion of bromine, instead of two, to one of amide nearly all of the amide was recovered unchanged. Prolonged heating of diphenylsuccinamide on the water bath with a solution of sodium hydroxide, of the same concentration as in the experiments with sodium hypobromite, caused no change in the amide.

Action of Alkaline Sodium Hypobromite on 1,2-Diphenylethylenediamine

α-Benzildioxime (10 gm.) was reduced with sodium and absolute alcohol according to the directions given by Feist (4) and Feist and Arnstein (5). When reduction was complete, the solution was diluted with water and acidified with hydrochloric acid. The alcohol was then distilled off. In addition to the sodium chloride which separated during the distillation, the residual liquid on cooling deposited 2.4 gm. of a crystalline solid. The filtrate on treatment in the usual way gave Feist's diphenylethylenediamine (m.p. 90–92° C.). On redissolving the 2.4 gm. of material in water and then making the solution alkaline, a base precipitated. On recrystallization it melted at 120° C. Its properties agree with those of the diphenylethylenediamine obtained by Grossmann (6) from amarin. Calcd. for $C_{14}H_{16}N_2$: N, 13.2%. Found: N, 13.3%.

By substituting each of these diphenylethylenediamines for diphenylsuccinamide, under the foregoing conditions with sodium hypobromite, ammonia and benzaldehyde were formed on warming. A gummy mass formed and on removal of this nothing further separated on acidification.

α-Phenyl-o-chlorocinnamonitrile, $ClC_6H_4CH : C(C_6H_5)CN$

This substance was made by condensing equimolecular proportions of *o*-chlorobenzaldehyde and benzyl cyanide, dissolved in twice their weight of alcohol, by means of a few drops of 50% caustic soda. The condensation product which is formed quickly was obtained in colorless needles after several recrystallizations from alcohol; m.p. 107° C. Calcd. for $C_{15}H_{10}NCl$: C, 75.12; H, 4.17; N, 5.84%. Found: C, 75.55; H, 4.40; N, 6.10%.

Attempted Preparation of o-Chlorodiphenylsuccinonitrile

The procedure was that which has been used here repeatedly for similarly constituted nitriles. α-Phenyl-*o*-chlorocinnamonitrile (24 gm.) was dissolved in 700 cc. of alcohol at 50–60° C. A solution of 27.4 gm. of potassium cyanide in 80 cc. of water was added and the temperature maintained in this range for an hour. A solution of acetic acid (12 gm. in 50 cc. water) was then introduced gradually at the bottom of the solution. The mixture was allowed to stand for three days. It became very dark brown but nothing separated. About 600 cc. of alcohol was removed by distillation and the residue diluted copiously with water. A large quantity of a brown spongy mass separated and from this it was not possible to obtain a pure substance by crystallization.

A portion (10 gm.) of this crude product was dissolved in warm 80% sulphuric acid and allowed to stand overnight. On diluting with water a precipitate formed. After it was digested with caustic soda, 2.5 gm. of insoluble material was left. This was recrystallized from a large volume of hot acetic acid and seemed to be *o*-chlorodiphenylsuccinamide. The recrystallized material softened at 278° and melted at 280° C. Calcd. for $C_{16}H_{15}O_2N_2Cl$: C, 63.5; H, 4.95; N, 9.25%. Found: C, 63.5; H, 4.85; N, 9.75%. The caustic soda solution gave a mixture of acids from which no single substance was isolated.

A second portion of 10 gm. of the crude product was dissolved in 90% sulphuric acid and, after dilution until the acid had a strength of about 70%, was heated at 120° C. for four hours. Further dilution gave a mixture of acidic substances from which, by recrystallization from alcohol, an acid, m.p. 217° C., was isolated ultimately in small yield. The analytical figures agree with those for *o*-chlorodiphenylsuccinic acid. Calcd. for $C_{16}H_{13}O_4Cl$: C, 63.1; H, 4.27%; equivalent, 152.3. Found, C, 63.1; H, 4.32%; equivalent 153.8.

Some later experiments performed with the assistance of Mr. J. G. Craig showed that α-phenyl-*o*-chlorocinnamonitrile reacts with hydrogen cyanide much faster than does α-phenylcinnamonitrile, and an attempt was made to isolate the addition compound by using milder conditions.

A hot solution of 9.6 gm. of α-phenyl-*o*-chlorocinnamonitrile in 100 cc. of alcohol was mixed with 5.2 gm. of potassium cyanide in 15 cc. of water and kept gently simmering for two hours. The mixture gradually acquired a deep brown color. On cooling and diluting with water a brown, amorphous precipitate (about 1 gm.) separated. The filtrate on acidification deposited 8 gm. of a crystalline precipitate. After several recrystallizations from hot benzene or hot dilute alcohol the substance was obtained in rhomboidal plates, m.p. 135° C. Although the melting point seems low, the analytical figures indicate that the substance is the half nitrile of *o*-chlorodiphenylsuccinic acid (presumably $C_6H_5 . CH(CN)C_6H_4Cl . CO_2H$, since diphenylsuccinonitrile is stable under similar conditions). Calcd. for $C_{16}H_{12}O_2NCl$: N, 4.95; Cl, 12.44%. Found: N, 4.95; Cl, 12.65%.

pp'-Dinitrodiphenylsuccinamide,

$$CONH_2 . CH(C_6H_4NO_2) . CH(C_6H_4NO_2) . CONH_2$$

Diphenylsuccinic nitrile (25 gm.) was dissolved in 275 cc. of 90% sulphuric acid on the water bath, cooled to room temperature and nitrated with efficient stirring with excess nitric acid. After standing four hours the mixture was poured on ice and the precipitated amide was washed with sodium carbonate. The yield at this stage was 30.2 gm. (80% of theoretical). The substance is very difficultly soluble in the usual organic solvents but was recrystallized by dissolving it in hot phenol and, after the solution had cooled, precipitating with acetone. The pure substance is faintly yellow, and melts at 294° C. with decomposition. Calcd. for $C_{16}H_{14}O_6N_4$: C, 53.6; H, 4.91; N, 15.64%. Found: C, 53.2; H, 3.28; N, 15.64. It gave, on hydrolysis with 90% sulphuric acid at 120° C., dinitrodiphenylsuccinic acid, m.p. 242° C., which was seemingly identical in properties with the acid described by Reimer (10). It was oxidized to *p*-nitrobenzoic acid by chromic acid dissolved in glacial acetic acid.

The action of alkaline sodium hypobromite on this amide, using the same conditions as with diphenylsuccinamide, brought about the evolution of ammonia as the amide dissolved. Acidification gave an amorphous precipitate from which a very small proportion of *pp'*-dinitrodiphenylsuccinamic acid was obtained. This substance after recrystallization from alcohol softened at 230° C. and gradually decomposed as the temperature was raised. Calcd. for $C_{16}H_{13}O_7N_3$: N, 14.6%; equivalent 359. Found: N, 15.0%; equivalent 359.

p-Methoxydiphenylsuccinonitrile

This substance was prepared by the method used by Lapworth and McRae (8) for diphenylsuccinonitrile. Later the slightly different method of Brand and Loehr was used with equally good results. Recrystallized from glacial acetic acid, the substance was obtained in fine, colorless needles; m.p. 204° C. Brand and Loehr give the melting point as 193° C. Calcd for $C_{17}H_{14}ON_2$: C, 77.9; H, 5.35; N, 10.68%. Found: C, 77.5; H, 5.36; N, 11.2%.

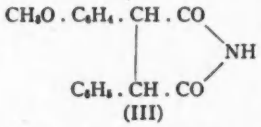
All attempts to hydrolyze this dinitrile to the corresponding diamide failed. Using sulphuric acid of various strengths, fuming hydrochloric acid or alcoholic

potash, the substance was in general either not attacked or else hydrolyzed to *p*-methoxydiphenylsuccinic acid. In some attempts with concentrated sulphuric acid sulphonation occurred, as observed by Brand and Loehr. These authors obtained *p*-methoxydiphenylsuccinic acid as needles from xylene; m.p. 221° C., whereas the writers' specimen of this acid, recrystallized from alcohol or acetic acid, melted at 227° C. Calcd. for $C_{17}H_{16}O_5$: C, 68.0; H, 5.3%. Found: C, 67.9; H, 5.1%.

Diethyl p-Methoxydiphenylsuccinate, $CH_3O \cdot C_6H_4 \cdot CH(CO_2C_2H_5) \cdot (C_6H_5)CH \cdot CO_2C_2H_5$. This was obtained by esterifying the foregoing acid with ethyl alcohol and sulphuric acid. Recrystallized from alcohol it melted at 102° C. Calcd. for $C_{21}H_{24}O_5$: C, 70.8; H, 6.7%. Found: C, 70.9; H, 6.8%. It was accompanied by the half ester, ethyl *p*-methoxydiphenylsuccinate, $CH_3OC_6H_4(CO_2Et)C_6H_5CHCO_2H$ (?) from which it was separated by means of sodium carbonate. The latter substance was recrystallized from alcohol; m.p. 154° C. Calcd. for $C_{19}H_{20}O_5$: C, 69.5; H, 6.1%; equivalent, 328. Found: C, 69.4; H, 5.8%; equivalent, 329.

p-Methoxydiphenylsuccinimide (III)

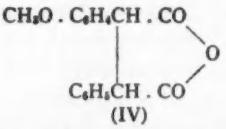
Ammonium *p*-methoxydiphenylsuccinate (10 gm.) was heated in a flask at 12 mm. until the residue began to char. The red gummy distillate was extracted with acetone, and from the acetone extract 6.1 gm. of crude imide was obtained. This on recrystallization from alcohol gave colorless minute needles, m.p. 178° C. It may also be recrystallized from benzene. Calcd. for $C_{17}H_{15}O_3N$: C, 72.2; H, 5.3; N, 5.0%. Found: C, 72.0; H, 5.5; N, 5.5%.



(III)

p-Methoxydiphenylsuccinic Anhydride (IV)

An attempt to prepare this substance by distilling *p*-methoxydiphenylsuccinic acid at 12 mm. gave a thick gummy distillate (soluble in ether) from which the desired anhydride could not be isolated.



(IV)

It was obtained easily by dissolving 10 gm. of the acid in 15 cc. of thionyl chloride with gentle warming. The excess thionyl chloride was distilled off and the residue recrystallized from benzene by the addition of petroleum ether. Star-shaped crystals melting at 114° C. were obtained. Calcd. for $C_{17}H_{14}O_3$: C, 72.3; H, 5.0%. Found: C, 72.8; H, 5.1%.

Action of Sodium Hypobromite on s-Diphenylsuccinimide

Diphenylsuccinimide was made from ammonium diphenylsuccinate according to the directions of Lapworth and McRae (8), but it was found that it could be made more readily by heating diphenylsuccinamide in a Claisen flask at 15 mm. pressure until the evolution of ammonia ceased. The distillate and the residue in the flask were thoroughly extracted with benzene and the diphenylsuccinimide thus obtained recrystallized from benzene. Twenty grams of amide gave 13 gm. of imide.

Diphenylsuccinimide (18.6 gm.), made into a thin paste with a little water, was stirred into a cold solution of sodium hypobromite prepared by dissolving 12 gm. of bromine in a solution of 15 gm. of sodium hydroxide in 75 cc. of water at −10° C. The imide did not appear to dissolve but formed a pasty mass. When the temperature had risen to 0° C., 9 gm. of sodium hydroxide was added and the mixture heated for three hours on the water bath at 80° C. The imide dissolved and ammonia was evolved. The solution, after cooling, was acidified with hydrochloric acid. The crude product obtained consisted chiefly of diphenylsuccinamic acid and diphenylmaleic anhydride. These were separated by heating with chloroform in which the former substance is insoluble. From 16 gm. of crude product, 9.3 gm. of the amido-acid and 6.2 gm. of diphenylmaleic anhydride were obtained.

Diphenylsuccinamic acid was recrystallized from ethyl alcohol. It softens at 215° C. and melts with decomposition at 218° C. Calcd. for $C_{16}H_{15}O_3N$: C, 71.4; H, 5.57; N, 5.20%; equivalent 269. Found: C, 70.6; H, 5.7; N, 5.36%; equivalent 270.5. Hydrolysis with 90% sulphuric acid gave diphenylsuccinic acid.

Diphenylmaleic anhydride was recrystallized from dilute alcohol in needles; m.p. 155° C. Calcd. for $C_{16}H_{12}O_2$: C, 76.8; H, 4.0%. Found: C, 76.2; H, 4.1%. The substance obtained agrees in properties with the description given by Reimer (10). Potassium permanganate oxidized it to benzoic acid, and chromic acid in acetic acid oxidized it to benzil. It reduced ammoniacal silver nitrate and was converted by ammonia into diphenylmaleic imide; m.p. 214° C. Calcd. for $C_{16}H_{11}O_2N$: C, 77.1; H, 4.4; N, 5.6%. Found: C, 77.3; H, 4.5; N, 5.9%.

Under the conditions used by Hoogewerff and van Dorp (7) in their experiments on succinimide, somewhat different results were obtained. Diphenylsuccinimide (12.5 gm.) was added to a cold hypobromite solution made from 8 gm. of bromine added to 16 gm. of sodium hydroxide in 150 cc. of water and then heated on the water bath. Ammonia was evolved and the product obtained on acidification (10.7 gm.) gave 5.2 gm. of diphenylmaleic anhydride when treated as before. No amido-acid could be isolated from the part insoluble in chloroform.

Action of Sodium Hypobromite on Diphenylsuccinamic Acid

Using the same conditions as with the amide, diphenylsuccinamic acid was treated with alkaline sodium hypobromite solution. The only substance that could be identified after acidification was benzoic acid.

1,2-Diphenylsuccinyl Chloride (V)

Diphenylsuccinic acid (12 gm.) was mixed intimately in a flask with 22.1 gm. of phosphorus pentachloride. Reaction occurred slowly and the mixture was warmed gently on the water bath until it had become quite gummy. Phosphorus oxychloride and unchanged phosphorus pentachloride were removed by heating on the water bath under reduced pressure. Attempts to distil the acid chloride

$$\begin{matrix} C_6H_5CH \cdot COCl \\ | \\ C_6H_5CH \cdot COCl \end{matrix}$$
(V)

under reduced pressure were unsuccessful. The residue was dissolved in hot benzene and recrystallized from the same solvent, from which the acid chloride was deposited as flaky colorless crystals; m.p. 190° C. It was not readily attacked by water but it was rapidly dissolved by hot alkali from which acids precipitated diphenylsuccinic acid. Calcd. for $C_{16}H_{12}O_2Cl_2$: C, 62.5; H, 3.91; Cl, 23.1%. Found: C, 62.6; H, 3.9; Cl, 23.4%. It was converted by grinding with ammonium carbonate into diphenylsuccinamide. On warming with ethyl alcohol the diethyl ester of mesodiphenylsuccinic acid, m.p. 139° C., was obtained. Anschütz and Bendix (1) give the melting point as 140–141° C. The acid chloride is therefore the symmetrical dichloride of β- or mesodiphenylsuccinic acid.

In some preparations the acid chloride was accompanied by a greenish-yellow substance which was separated by dissolving it in ether. The substance was identified as diphenylmaleimide and evidently arose from the presence of diphenylsuccinamic acid in the diphenylsuccinic acid used. By digesting diphenylsuccinamide (3 gm.) with phosphorus pentachloride it was not found possible to isolate diphenylmaleimide, but by warming the crude product with dilute caustic soda and acidifying, 2 gm. of diphenylmaleic anhydride was obtained.

Acknowledgments

The authors express their thanks to Messrs. F. S. Wilder and W. R. Conn who assisted in the experimental work.

References

1. Anschütz, R. and Bendix, P. Ann. 259 : 61-100. 1890.
2. Brand, K. and Loehr, O. J. prakt. Chem. 109 : 359-375. 1925.
3. Conn, W. R. M. A. Thesis, Queen's University. (Unpublished.) 1933.
4. Feist, F. Ber. 27 : 213-215. 1894.
5. Feist, F. and Arnstein, H. Ber. 28 : 3167-3181. 1895.
6. Grossmann, G. Ber. 22 : 2298-2305. 1889.
7. Hoogewerff, S. and van Dorp, W. A. Rec. trav. chim. 10 : 4-12. 1891.
8. Lapworth, A. and McRae, J. A. J. Chem. Soc. 121 : 1699-1712. 1922.
9. McRae, J. A. and Vining, W. H. Can. J. Research, 6 : 409-416. 1932.
10. Reimer, C. L. Ber. 14 : 1802-1808. 1881.
11. Weidel, H. and Roithner, E. Monatsh. 17 : 172-190. 1896.

THE PREPARATION AND PHYSICAL PROPERTIES OF ALIPHATIC ACETYLENES[1]

By F. R. Morehouse[2] and O. Maass[3]

Abstract

This paper deals with the preparation and determination of the physical properties of the lower members of the acetylene series—methyl, ethyl, dimethyl and propyl acetylenes. A comprehensive determination of the physical properties of these compounds has been made and the results have been interpreted along with similar data obtained for the paraffin and olefine series. The acetylenes have been found to differ from the paraffins and olefines. The difference is attributed to the polarity of the acetylenes as a group. This is further substantiated by a comparison of the properties of the two isomeric acetylenes, dimethyl and ethyl acetylenes.

Introduction

A systematic study of the physical properties of the paraffin and the olefine hydrocarbons has been the subject of investigation in this laboratory. Those hydrocarbons containing two and three carbon atoms were investigated by Wright and Maass (10), and the properties of the four-carbon paraffins and olefines were determined by Coffin and Maass (3). The physical constants of the first four-carbon acetylene have been given in a previous publication accompanied by a redetermination of the properties of methyl acetylene (11). In this paper the physical constants of dimethyl acetylene will be added to those already determined for the other acetylenes and a comparison made, on the basis of these physical properties, of the paraffin, olefine and acetylene series from the point of view of their molecular structure.

Experimental

In order to obtain the acetylenes in the highest state of purity they were prepared by direct synthesis from the simple constituents of each compound. This method, with certain modifications, was that used originally by Lebeau and Picon (8) and consists in the alkylation of sodium acetylide in liquid ammonia as a reaction medium. Since this method and modifications have been previously described in detail (11) they will not be repeated. The preparation of propyl acetylene was carried out in the usual manner. Dimethyl acetylene was prepared by the methylation of the sodium derivative of methyl acetylene in a manner analogous to that used in the preparation of the other alkyl acetylenes. A large quantity of methyl acetylene was prepared and passed into the blue solution of sodium in liquid ammonia. Disappearance of the blue color showed complete formation of sodium methyl acetylide. Lebeau (9) found this reaction to take place in the following way:

$$3CH_3C{\equiv}CH + 2Na = 2CH_3C{\equiv}CNa + CH_3CH{=}CH_2$$

[1] *Manuscript received July 14, 1934.*
Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada, with financial assistance from the National Research Council of Canada.
[2] *Holder of a studentship under the National Research Council of Canada at the time during which part of the investigation was carried out.*
[3] *Macdonald Professor of Physical Chemistry, McGill University.*

When all the sodium had reacted with the methyl acetylene, the ammonia and propylene were allowed to pass off, leaving the white, solid sodium derivative behind. As the mercury and silver derivatives of methyl acetylene are non-explosive (6, 7) this procedure was considered safe. Methylation was then carried out and the dimethyl acetylene separated in the usual manner. As there were discrepancies in the results for the parachor, compared with those for the other acetylenes, it was thought advisable to prove the identity of the dimethyl acetylene obtained in this way.

By determining the vapor density of the product the molecular weight was found to agree with the theoretical value for dimethyl acetylene within 1%. As the boiling point of the product was found to be 27.1° C., which agrees with the values found by other investigators (1), 27.2° and 27.6° C., it was not considered likely that the product could be one of the isomers, 1, 2-butadiene and 1, 3-butadiene, since the boiling points are given in the International Critical Tables as 19.0° C. for the former and −2.6° C. for the latter. The boiling point of the isomeric ethyl acetylene has been found to be 8.6° C. As a final check, however, the compound was brominated and analyzed. The melting point of the brominated compound, 210° C., agrees reasonably with the value determined by Faworsky (4) and is quite different from the melting points of the bromination products of possible isomers (2). The results of analysis gave 86.6% bromine, as compared to the theoretical 85.5%.

Final purification of all compounds, after they had been separated from ammonia, was effected by fractional distillation (3). The final, pure fractions were then distilled into the apparatus used for the determination of the different physical properties, such as density, melting point, surface tension, etc.

Physical Properties of the Hydrocarbons

The temperature baths used in the determination of the densities, surface tensions, vapor pressures and melting points consisted of a liquid, suitable for the temperature range under which the observations were being carried out, contained in a transparent Dewar flask. All baths were kept vigorously stirred with a current of dry air bubbles, and temperatures were measured with a standard thermometer to 0.1° C. Solid carbon dioxide was used as the cooling agent from 0 to −78° C. and the bath over this temperature range consisted of acetone or ether. For the measurement of properties at lower temperatures high test gasoline, cooled by means of liquid air dropped into a Pyrex test tube immersed in the bath, was used.

The data for methyl and ethyl acetylenes have already been given (11). In Figs. 1, 2 and 3, however, are shown, for purposes of comparison, a graphical representation of the various properties of methyl, ethyl, propyl and dimethyl acetylenes.

Density

Density determinations were made by the dilatometer method described by Wright and Maass (10). The dilatometer was filled by distilling the pure

compound into it, thus obviating the possibility of any contamination by impurities. All the pieces of apparatus used in determining the different physical properties were filled in the same way. In Fig. 1 will be found the densities of dimethyl and propyl acetylenes.

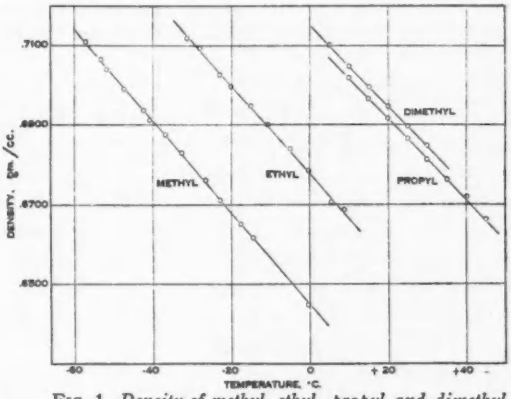


FIG. 1. *Density of methyl, ethyl, propyl and dimethyl acetylenes.*

Surface Tension

The surface tensions of the acetylenes were determined by the capillary rise method. A description of this method and the accuracy obtainable are to be found in the paper referred to above (10). The curves derived for all the surface tensions are to be found in Fig. 2.

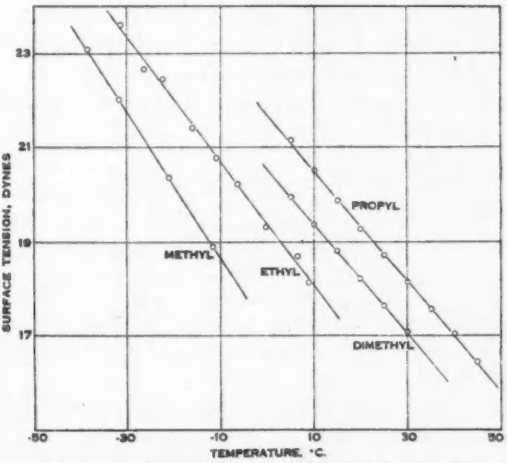


FIG. 2. *Surface tension of methyl, ethyl, propyl and dimethyl acetylenes.*

Melting Points

Melting points were determined by freezing the hydrocarbons in small bulbs and allowing the bath to warm up slowly. The temperature taken as the melting point was halfway between the temperature at which melting began and that at which the solid just disappeared. In all cases a sharp melting point was observed. The melting points are listed with other properties in Table I.

Vapor Pressures

Vapor pressure curves for the hydrocarbons whose boiling points were below room temperature were determined in the fractionating apparatus. For those compounds having boiling points about or above room temperature a small bulb containing the hydrocarbon was sealed to a manometer, and both were immersed in a bath whose temperature could be regulated. This avoided condensation in the connecting tubing and manometer. In Fig. 3 will be found vapor pressure curves of the four hydrocarbons.

TABLE I

PHYSICAL PROPERTIES OF THE FIRST THREE OR FOUR MEMBERS OF THE PARAFFINS, OLEFINES AND ACETYLENES

Compound	M.p., °C.	B.p., °C.	*M.L.H.*	D_b	S_b	Total *S.E.*	*K* (*R.S.*)	$\frac{M.L.H.}{T_b}$	t_c Calc.	$\frac{T_b}{T_c}$	V_m	Parachor		
												Obs.	Calc.	Diff., %
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ethane	−172.0	−88.3	3880	.5469	16.05	45.7	1.98	21.0	34.6	.60	54.9	110.4	112.2	1.6
Propane	−189.9	−44.5	4700	.5853	15.63	50.3	2.15	20.6	91.6	.62	75.0	150.6	151.2	0.4
n-Butane	−135.0	− 0.5	5597	.6014	14.90	47.8	2.21	20.5	147.0	.64	96.3	190.6	190.2	0.2
i-Butane	−145.0	−10.2	5480	.5944	14.10	47.2	2.23	20.8	129.5	.64	97.6	188.8	190.2	0.2
Ethylene	−169.4	−103.9	3510	.5699	16.50	47.7	2.14	20.8	5.9	.60	49.1	99.4	101.2	1.8
Propylene	−185.2	−47.0	4600	.6095	16.70	49.7	2.12	19.0	91.3	.62	69.0	139.7	140.2	0.4
α-Butylene	−190.0	− 6.1	5400	.6250	15.95	51.1	2.21	20.2	145.0	.64	89.5	179.9	179.2	0.4
β-Butylene	−127.0	1.0	5400	.6300	16.78	51.2	2.14	19.7	156.0	.64	88.9	178.7	179.2	0.3
γ-Butylene	−146.8	− 6.6	5200	.6268	15.72	49.8	2.15	19.7	147.0	.64	89.3	178.4	179.2	0.4
Acetylene	− 81.0	−83.6	4273	.6208	19.58	56.0	2.02	22.2	39.7	.61	41.9	88.1	90.4	2.6
Methyl acetylene	−101.5	−23.3	5562	.6698	20.70	59.4	1.99	22.3	121.6	.62	59.7	127.5	129.4	1.5
Ethyl acetylene	−122.5	8.6	5983	.6682	18.30	53.5	1.96	21.3	190.5	.61	80.8	167.2	168.6	0.8
Dimethyl acetylene	− 24.0	27.1	6434	.6873	17.40	51.8	1.75	21.4	215.5	.61	78.6	160.7	168.6	4.6
Propyl acetylene	− 98.0	39.7	7087	.6718	17.00	53.3	2.12	22.6	220.3	.63	101.3	206.2	207.2	0.5

NOTE:—*M.L.H., molecular latent heat of evaporation; D_b, density at boiling point; S_b, surface tension at the boiling point; S.E., surface energy; K (R.S.), Ramsay and Shields constant; T_b, b.p. °A; t_c, critical temperature °C.; T_c, critical temperature °A; V_m, molecular volume.*

The latent heats of evaporation are given in Table I. They were calculated from the vapor pressure curves by means of the Clapeyron equation. Boiling points are also given in Table I.

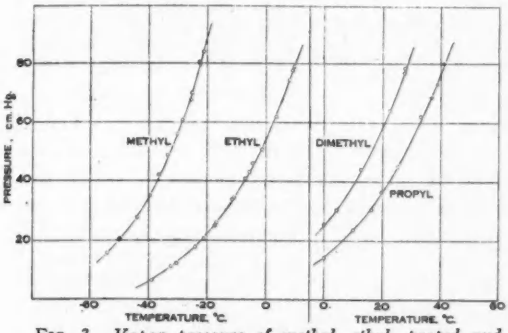


FIG. 3. *Vapor pressure of methyl, ethyl, propyl and dimethyl acetylenes.*

Discussion

The constants determined for all the hydrocarbons investigated in this laboratory are compiled in Table I. In the discussion of the acetylenes the properties will, in general, be taken up in the order in which they appear in the table.

It is seen that the melting points of the acetylenes are, for compounds containing the same number of carbon atoms, higher than those of either the paraffins or olefines. Unsaturation in the acetylenes apparently accounts for their higher melting points, and furthermore symmetry of the molecule seems to play an important role in governing this property, as may be seen in the case of the isomers ethyl acetylene and dimethyl acetylene.

It will be observed, when the more additive boiling points (Column 2), molecular latent heat of evaporation (Column 3) and critical temperature (Column 9), are compared, that for compounds containing the same number of carbon atoms there is a decrease in all these properties in the paraffin and olefine series as compared with the acetylene series. However, for the acetylenes that are still lower in molecular weight, these values are distinctly higher than those for their corresponding compounds in the other two series. This apparent anomaly makes it possible to classify the acetylenes in a group by themselves, separate from the paraffins and olefines. This is more evident as other properties are considered and is due to the fact that the acetylene linkage is of a more definitely pronounced polar character.

That all these substances are in the liquid state, in an unassociated normal condition, is shown by the values of the Ramsay and Shields constants (Column 7), although the values obtained for the acetylenes seem to be consistently low.

When considering the total surface energy (Column 6) in relation to the first few members of an homologous series, two types of compounds may be separately classified. There is first that in which no polar group is attached to the molecule, such as the paraffins and olefines listed above. Second, there is a large number of compounds such as the alcohols and fatty acids which possess a highly polar group attached to one end of the molecule.

As can be seen from the data assembled in Table I, the total surface energies of those compounds belonging to the first class increase slightly until a more or less limiting value is reached.

In compounds of the second type an altogether different result is observed. Several homologous series of this kind have been investigated and Hunten and Maass (5) found, when studying an homologous series of fatty acids, that the total surface energy falls off very rapidly as the series is ascended and reaches a normal value for all the higher members of the series. This same result has been found for other series whose members contain a polar group.

When the total surface energies of the acetylenes are compared it is seen at once that they follow this general tendency. This evidence points to the fact, not encountered in the paraffin and olefine series, that the acetylene linkage, a triple bond, exerts a decidedly polar influence on the molecule.

In general the more symmetrical the molecule the greater is its attraction for molecules of the same species. Thus it would be expected that dimethyl acetylene, being the symmetrical isomer of ethyl acetylene, would have a higher surface tension at the boiling point (Column 5). This theory is apparently not supported by the data obtained for the acetylenes. However, in this particular case the molecule is symmetrical around a group that is highly polar, compared to the polarity of the olefines, and since the property of surface tension is particularly sensitive to this factor it may well be due to this that the symmetrical dimethyl acetylene has a smaller surface tension at the boiling point.

Consequently all properties depending on surface tension, owing to the symmetry of the molecule, *i.e.*, the shielding effect of the hydrogen atoms surrounding the triple bond, are lower for dimethyl acetylene than for ethyl acetylene. As will be seen later this symmetry seems also to make the molecule appear less unsaturated. Thus it is not surprising to find that the parachor, which is directly related to surface tension, should have a value considerably lower for the symmetrical isomer and also lower than the theoretical value, which does not take into account certain "strain" factors apparently caused by symmetry of the molecule.

Confining the discussion to an empirical conception of these compounds, the acetylenes, and dimethly acetylene, in particular, may be considered from another point of view. If the molecular volume at the boiling point (Column 11) is considered to give a comparative value of the actual volume of the molecules, then this volume has been found to be an additive and constitutive property from the point of view of definite values assigned to the elements and their mode of linkage. Thus carbon and hydrogen in all organic compounds have fairly definite values where the carbon has four ordinary linkages. Unsaturation requires the addition of a constant value depending on whether the unsaturation is of an ethylene or acetylene type. The latter has the larger value, and the greater the extent of unsaturation the greater is the difference between the molecular volume of the compound and that given by the straight additive values of the atoms of the elements involved.

The molecular volumes of the acetylenes, using Kopp's constants where C=2H=11, are given in Table II and compared with their experimental values determined from the density at the boiling point.

TABLE II

MOLECULAR VOLUMES OF THE ACETYLENES

—	Kopp's	Experimental	Diff.
Acetylene	33.0	41.9	8.9
Methyl acetylene	55.0	59.7	4.7
Ethyl acetylene	77.0	80.8	3.8
Dimethyl acetylene	77.0	78.6	1.6
Propyl acetylene	99.0	101.3	2.3

It is seen that the difference in volume caused by the triple bond is greatest for acetylene and falls off rapidly as the series is ascended. That the value for dimethyl acetylene should be much less than that for the unsymmetrical isomer is indicative of the fact that the molecule of dimethyl acetylene is rendered less unsaturated by the symmetry of its structure.

This evidence, obtained from an entirely empirical point of view, agrees with, and moreover substantiates, the same conclusions drawn from a consideration of the calculated values obtained from surface tension measurements.

Finally, it may be said that all the properties of dimethyl acetylene, in relation to its own series and to members of the paraffin and olefine series, point to the fact that its behavior is greatly influenced by its polarity and molecular strain. All the properties that would be influenced by polarity of the molecule are seen to manifest this characteristic in the acetylene series by their divergence from the general tendencies observed in the paraffin and olefine series.

In conclusion, the acetylenes as a class of compounds stand out separately and distinctly from the paraffins and olefines, as far as the physical properties and their interpretation are concerned.

References

1. Annalen of Russian Journal of Physical Chemistry, 13 : 392.
2. Beilstein's Handbuch der organischen Chemie. J. Springer, Berlin, 1 : 122. 1918.
3. COFFIN, C. C. and MAASS, O. J. Am. Chem. Soc. 50 : 1427-1437. 1928.
4. FAWORSKY, A. J. prakt. Chem. 42 : 143-149. 1890.
5. HUNTEN, K. W. and MAASS, O. J. Am. Chem. Soc. 51 : 153-165. 1929.
6. KUTSCHEROFF, M. Ber. 17 : 13-29. 1884.
7. LIEBERMANN, C. Ann. 135 : 266-290. 1865.
8. LEBEAU, P. and PICON, M. Compt. rend. 156 : 1077-1079. 1913.
9. LEBEAU, P. and PICON, M. Compt. rend. 157 : 137-139. 1913.
10. MAASS, O. and WRIGHT, C.H. J. Am. Chem. Soc. 43 : 1098-1111. 1921.
11. MOREHOUSE, F. R. and MAASS, O. Can. J. Research, 5 : 306-312. 1931.

β-[3-NITROPHENYL]-β-[ANTHRON-(9)-YL-(10)]-PROPIONIC ACID AND ITS DERIVATIVES[1]

By Alexandre Vachon[2], Paul E. Gagnon[3] and John Kane[4]

Abstract

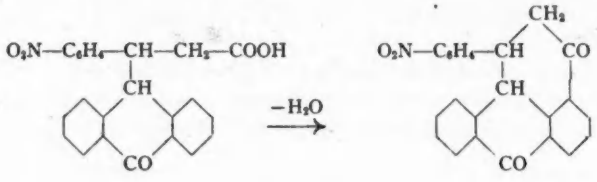
β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid, unlike β-phenyl-β-[anthron-(9)-yl-(10]-propionic acid (2, 3), was not found to undergo cyclisation to form a hydrindone derivative when treated with aluminium chloride. Treatment with concentrated sulphuric acid caused the formation of β-[3-nitrophenyl]-β-[(10)-oxy-anthron-(9)-yl-(10)]-propionic acid. The lactone of this acid was then formed together with Bz-1′-oxy-3′(3-nitrophenyl)-1-9-benzanthrone.

The following compounds have been prepared, as far as the authors are aware, for the first time: 3-nitrobenzylidene-dimethyl-malonate; the dimethyl and diethyl esters of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic acid; β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid; the chloride and ethyl ester of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid; β-[3-nitrophenyl]-β-[(10)-oxy-anthron-(9)-yl-(10)]-propionic acid and its lactone; and Bz-1′-oxy-Bz-3′(3-nitrophenyl)-1-9-benzanthrone.

Introduction

In previous papers (2, 3) it has been shown that by cyclisation of β-phenyl-β-[anthron-(9)-yl-(10)]-propionic acid or its chloride, hydrindone derivatives can be prepared.

Theoretically, it might be expected that β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid (I), or its chloride, would not give rise to hydrindone derivatives, owing to the presence of the nitro group, since it is difficult to bring about cyclisation of nitropropionic acid (7). The authors were of the opinion that a benzanthrone derivative (II) would be formed instead (1).



(I) (II)

Accordingly, β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid and its chloride, which have not hitherto been described, were prepared by a method similar to that used in the preparation of β-phenyl-β-[anthron-(9)-yl-(10)]-propionic acid (2, 3). The acid and its chloride were then treated with sulphuric acid and with aluminium chloride in attempts to bring about cyclisation.

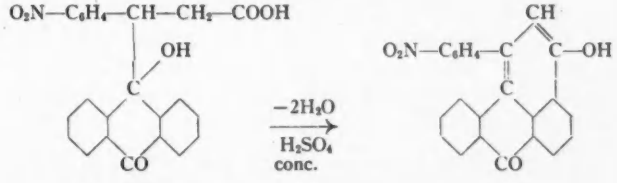
[1] *Manuscript received August 23, 1934.*
Contribution from the "Ecole Supérieure de Chimie", Laval University, Quebec, with assistance from the National Research Council of Canada. This paper is based on a thesis presented by John Kane in partial fulfilment of the requirements for the degree of D.Sc. at Laval University.
[2] *Director of the "Ecole Supérieure de Chimie", Laval University.*
[3] *Professor of Chemistry, Laval University.*
[4] *Graduate Student, Laval University.*

The acid was obtained by condensation of 3-nitrobenzaldehyde with dimethyl-malonate, and by further condensation of the product with anthrone. The dimethyl ester of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic acid thus formed gave rise, on hydrolysis, to β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid, which, on treatment with phosphorus pentachloride, yielded the chloride. The diethyl ester, which is cheaper, may also be used.

β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionyl chloride is a white solid; m.p. 147° C. It is much more stable than the corresponding β-phenyl-β-[anthron-(9)-yl-(10)]-propionyl chloride. When dissolved in anhydrous carbon disulphide and treated with pure aluminium chloride, it does not give rise to hydrindone derivatives, as does the other; it remains unaltered. The nitro group in the para position renders the elimination of a molecule of hydrogen chloride more difficult.

Nitrobenzene was also used as solvent, but on heating the solution to temperatures greater than 100° C., hardly any hydrogen chloride was evolved, and a large quantity of resins was formed. Benzene was not used as solvent as it was assumed that this chloride, like β-phenyl-β-[anthron-(9)-yl-(10)]-propionyl chloride, would react with this solvent.

β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid is more stable than β-phenyl-β-[anthron-(9)-yl-(10)]-propionic acid. With sulphuric acid, it was impossible to obtain hydrindone derivatives or the expected benzanthrone derivative. Two other products were formed, however—a lactone and an oxy-benzanthrone (IV). In order to effect cyclisation, it was necessary to heat the sulphuric acid mixture at 150° C., and there was then a strong evolution of sulphur dioxide. β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid was oxidized to β-[3-nitrophenyl]-β-[(10)-oxy-anthron-(9)-yl-(10)]-propionic acid (III), as it contains an easily oxidizable tertiary carbon (6). The oxy-acid immediately lost one or two molecules of water to give rise to the lactone or to the oxy-benzanthrone.



(III) (IV)

The oxy-acid was easily prepared by oxidizing β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid at room temperature with the calculated amount of potassium permanganate, and also by hydrolysis of the lactone. When treated with concentrated sulphuric acid the oxy-acid yielded the lactone and Bz-1′-oxy-Bz-3′(3-nitrophenyl)-1-9-benzanthrone.

The constitution of the lactone was established by hydrolyzing it, and also by oxidizing it with potassium permanganate in alkaline solution. The only products obtained were 3-nitrobenzoic acid and anthraquinone.

Bz-1′-oxy-Bz-3′(3-nitrophenyl)-1-9-benzanthrone, oxidized under the same conditions, did not yield anthraquinone, but gave rise to acidic products. The oxy-benzanthrone is a red compound which can exist in two tautomeric forms.

It is soluble in alcohol and is yellow in acid solution and violet in alkaline solution. It is a good indicator; the change in color from violet to yellow occurs when the pH value is 4.8 (determined with hydrogen electrode).

Experimental

3-Nitrobenzylidene-malonic and β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic Esters

3-Nitrobenzylidene-dimethyl-malonate. The reaction was carried out at room temperature, as Knoevenagel's method (4) for the 4-nitro compound gave lower yields. Dimethyl-malonate (22 gm.), and 3-nitrobenzaldehyde (26.4 gm.), were placed in a flask, which was well stoppered, and left for nine days at room temperature. After three days the solution was clear. Later 3-nitrobenzylidene-dimethyl-malonate separated slowly, and after nine days the contents of the flask was solid. After crystallization from alcohol and washing with ether, 33 gm. of 3-nitrobenzylidene-dimethyl-malonate was obtained; m.p., 99–100° C.; (theory, 44.1 gm.). Yield, 75%. Calcd. for $C_{12}H_{11}O_6N$: C, 54.31; H, 4.18; N, 5.28%. Found: C, 53.79; H, 4.21; N, 5.15%.

3-Nitrobenzylidene-diethyl-malonate. This substance was readily obtained by Kötz's method (5), by condensing, in the presence of piperidine, equimolecular quantities of 3-nitrobenzaldehyde and diethyl-malonate. From 125.8 gm. of diethyl-malonate, 186.9 gm. of 3-nitrobenzylidene-diethyl-malonate was obtained; m.p., 75–76° C. Yield, 81%.

Dimethyl ester of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic acid, $O_2N \cdot C_6H_4 \cdot CH(C_{14}H_9O)CH(COOCH_3)_2$. Anthrone (8 gm.), crystallized from benzene and free from acetic acid, 12 gm. of 3-nitrobenzylidene-dimethyl-malonate, 80 cc. of anhydrous methyl alcohol and few drops of piperidine were heated in a flask, provided with a calcium chloride tube, on the water bath for five to six hours.

After cooling, the solid mass was filtered and washed with a little methyl alcohol. The ester was then almost pure. It crystallizes from acetic acid in colorless lens-shaped crystals; m.p., 188–189° C. Yield, 16 gm. (theory, 19 gm.), *i.e.*, 84%.

The ester is slightly soluble in methyl and ethyl alcohols and fairly soluble in acetic acid. Concentrated sulphuric acid dissolves it, forming a reddish solution which turns violet after a few days. Calcd. for $C_{26}H_{21}O_7N$: N, 3.05%. Found: N, 3.08%.

Diethyl ester of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic acid, $O_2N \cdot C_6H_4 \cdot CH(C_{14}H_9O) \cdot CH(COOC_2H_5)_2$. Anthrone (31.5 gm.), free from

acetic acid, 50 gm. of 3-nitrobenzylidene-diethyl-malonate and 80 cc. of anhydrous methyl alcohol were heated in a flask (reflux condenser) on a water bath, and a few drops of piperidine were added. The anthrone dissolved rapidly; 10 min. later, a few additional drops of piperidine were added and the flask was left on the water bath for another half hour.

The hot solution was rapidly filtered with suction and cooled to 0° C. On stirring, the product crystallized. It was filtered off, and washed with ether. The product was colorless. From 189 gm. of anthrone (six preparations), 393.5 gm. of the diethyl ester of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic acid was obtained. Yield, 83%. Recrystallized from methyl alcohol, it melted at 135–137° C. The ester is soluble in benzene, ether and acetic acid. Calcd. for $C_{28}H_{25}O_5N$: N, 2.87%. Found: N, 3.03%.

β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic Acid

Preparation from the dimethyl ester (10). The dimethyl ester of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic acid (10 gm.), 30% sulphuric acid (58 cc.) and glacial acetic acid (23 cc.) were heated in a flask (reflux condenser) for eight days at 120–125° C. After cooling, the mixture was poured into 300 cc. of water. The insoluble acid was filtered off, pulverized and washed with water; 8 gm. of a yellowish product was obtained. It was dissolved in a hot solution of sodium bicarbonate and, after cooling, the solution was filtered and acidified with dilute sulphuric acid. The precipitated acid was filtered off, washed with water and recrystallized from acetic acid.

The acid is readily soluble in concentrated ammonia, fairly soluble in methyl alcohol and acetic acid and slightly soluble in ethyl alcohol. From ethyl alcohol the pure acid crystallized in colorless rectangular crystals; m.p., 206–207° C.

The acid can be readily oxidized with potassium permanganate in alkaline solution, as will be shown below. It is fairly stable towards alkalies. Treatment with a boiling solution of potassium hydroxide (30%) merely dissolves it. Calcd. for $C_{23}H_{17}O_5N$: C, 71.29; H, 4.42; N, 3.61%. Found: C, 71.16; H, 4.41; N, 3.60%.

Preparation from the diethyl ester. The diethyl ester of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic acid (80 gm.), 30% sulphuric acid (430 cc.) and glacial acetic acid (172 cc.) were heated to boiling in a flask (reflux condenser). The ester melted and separated into small drops. After heating for three days the droplets solidified. The mixture was boiled for another day. (It was noted that if heating was prolonged (six or seven days) an impure acid, which could not be purified by many crystallizations from acetic acid, was obtained.) After cooling, the contents of the flask was poured into water and the insoluble acid was filtered off, pulverized, washed with water and dissolved in a hot saturated solution of sodium bicarbonate. After cooling, the solution was filtered and acidified with dilute sulphuric acid. The precipitated acid was filtered off, crystallized from acetic acid and washed with alcohol; m.p., 206–207° C. Yield, 45 gm. (74%).

This acid was proved identical with that obtained by hydrolysis of the dimethyl ester of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic acid by a mixed melting point determination.

β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionyl chloride, $O_2N \cdot C_6H_4 CH(C_{14}H_{19}O)CH_2 \cdot COCl$. Pure β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid (30 gm.), phosphorus pentachloride (17 gm.) and anhydrous carbon disulphide (200 cc.) were boiled in a flask, fitted with a reflux condenser and a calcium chloride tube, for two hours. The acid did not dissolve, but there was a strong evolution of hydrogen chloride, and crystals of the chloride were deposited; the unchanged acid remained uniformly dispersed in the solvent. After cooling, the chloride was filtered off and washed with anhydrous carbon disulphide. Yield, 29.3 gm. (93%). The chloride is slightly soluble in carbon disulphide, which is the best solvent for purifying it, the chloride being practically insoluble in most organic solvents. It forms colorless prisms; m.p., 147° C. Calcd. for $C_{23}H_{16}O_4NCl$: Cl, 8.74%. Found: Cl, 8.64%.

Ethyl ester, $O_2N \cdot C_6H_4 \cdot CH(C_{14}H_9O)CH_2 \cdot COOC_2H_5$. β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionyl chloride (5 gm.) and anhydrous ethyl alcohol (80 cc.) were boiled in a flask (reflux condenser) for 1½ hr. and left at room temperature overnight. The alcohol was distilled off and the residue dissolved in ether. On evaporation of the solvent, the ethyl ester crystallized out. It was recrystallized from alcohol and obtained in colorless prisms; m.p., 107–109° C. Yield, 4.5 gm. (88%). Calcd. for $C_{23}H_{21}O_5N$: N, 3.37%. Found: N, 3.28%.

β-[3-Nitrophenyl]-β-[(10)-oxy-anthron-(9)-yl-(10)]-propionic Acid Lactone

Preparation. Dried and well pulverized β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid (25 gm.) and concentrated sulphuric acid (250 cc.) were heated carefully in a flask and well shaken. The acid dissolved, forming a yellowish solution which became reddish at 115° C. and violet at 140° C. The mixture was heated to 150° C. and kept at that temperature for five minutes. Sulphur dioxide was evolved.

The hot solution was poured on to cracked ice. A brownish product precipitated. The mixture was diluted to three litres with water, heated to about 80° C. to coagulate the solid, cooled to room temperature, and filtered. The acid filtrate gave a dark violet color with alkalies. This color was due, as will be shown later, to the presence of a trace of Bz-1′-oxy-Bz-3′(3-nitrophenyl)-1-9-benzanthrone. The strongly colored product which remained on the filter paper was dried. It weighed 23.8 gm. Many extractions were made with 800-cc. portions of hot chloroform. Some of the solid dissolved. The dried residue (about 7 gm.) was identified as β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid. The chloroform solution was left to cool, and about 1 gm. of red crystals separated. They were filtered off and dried. They will be described later.

The filtrate was evaporated to dryness and 15 gm. of a product, still slightly colored by a trace of the red compound, was obtained (theory, 24.8 gm.).

Yield, 60%. If account is taken of the unaltered propionic acid (7 gm.), the yield was 88%. After crystallizing once from acetic acid and washing once with ether, the product was white and melted at 267–268° C.

The temperature and the time of heating employed seem to be the optimum for obtaining the best yield. In order that the lactone be formed, it is necessary to heat at a temperature of at least 140° C. and if the temperature is raised to 150° C., the time of heating must not be long. For instance, when the heating period was 12 min., the yield was only 46%, and when 30 min., carbonization took place. Many preparations were made under different conditions, not only to obtain a large quantity of the lactone, but also in attempts to increase the yield of the red compound, but only a very small quantity of the red substance was formed each time.

The lactone is slightly soluble in most of the organic solvents. It is insoluble in ether, carbon tetrachloride, carbon disulphide and methyl alcohol. It is very slightly soluble in ethyl alcohol, and somewhat more soluble in chloroform, toluene and acetic acid. The lactone is very readily decomposed by caustic alkalies, yielding anthraquinone. However, it was possible to hydrolyze it with a very dilute solution of potassium hydroxide, and so obtain the corresponding β-[3-nitrophenyl]-β-[(10)-oxy-anthron-(9)-yl-(10)]-propionic acid, as shown below. Calcd. for $C_{23}H_{15}O_5N$: C, 71.66; H, 3.92%. Found: C, 71.52; H, 3.90%.

Bz-1'-oxy-Bz-3'(3-nitrophenyl)-1-9-benzanthrone

In the preparation of the lactone, which was just described, traces of the red compound were separated by filtration. Analysis indicated the formula $C_{23}H_{13}O_4N$. From its properties, the oxidization products and its formation from the oxy-acid, it is believed to be a benzanthrone derivative which can exist in two tautomeric forms.

The red compound is fairly soluble in ether, acetic acid and alcohol. From the last two solvents it separates in crystals of the shape of a convex lens; m.p., about 305° C. With concentrated sulphuric acid, a violet solution (red by transmitted light) is obtained, which turns yellow when water is added. Its value as an indicator has been mentioned in the introduction. Calcd. for $C_{23}H_{13}O_4N$: C, 75.18; H, 3.57%. Found: C, 74.80; H, 3.51%.

Oxidation of the Lactone with Potassium Permanganate

The lactone (5 gm.), pulverized potassium hydroxide (3.4 gm.) and water (200 cc.) were heated in a flask (reflux condenser) on the water bath and 9.5 gm. of potassium permanganate was slowly added. At first the permanganate was reduced fairly rapidly, but after three hours the solution was only very slowly decolorized. The flask was left on the water bath for about 17 hr. The hot mixture was filtered with suction. The residue was washed with hot water, treated with dilute hydrochloric acid and extracted with chloroform. The solution was washed with water and dried with anhydrous calcium chloride. The solvent was then distilled off; 2 gm. of a yellowish product remained. The product separated from acetic acid in long needles; m.p.,

277–278° C. It was identified as anthraquinone by a mixed melting point determination. The filtrate was evaporated to small volume, acidified with dilute hydrochloric acid and extracted many times with ether. The ether was distilled off and about 2 gm. of a resinous product was obtained. It was very soluble in ether and alcohol and very slightly soluble in petroleum ether and benzene. It was difficult to purify it by crystallization. When heated carefully, some sublimed and formed long colorless needles. These were soluble in hot water and, after one crystallization from water, melted at 140–141° C. This substance was proved identical with 3-nitrobenzoic acid by a mixed melting point determination and by analysis. Calcd. for $C_7H_5O_4N$: C, 50.28; H, 3.01%. Found: C, 50.32; H, 3.12%.

Oxidation Products of Bz-1'-oxy-Bz-3'(3-nitrophenyl)-1-9-benzanthrone

Only a very small quantity of the oxy-benzanthrone was obtained in the preparation of the lactone. However, it was possible to prove that when the oxy-benzanthrone was oxidized under the same conditions as those used in the oxidation of the lactone, anthraquinone was not formed. The largest portion of the product obtained was acid in nature, indicating that it was a benzanthrone derivative. It was impossible to carry the study of the oxidation products of this red compound any further.

β-[3-Nitrophenyl]-β-[(10)-oxy-anthron-(9)-yl-(10)]-propionic Acid

(a) Preparation from the acid. β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid (25 gm.), potassium hydroxide (6 gm.), potassium permanganate (6.8 gm.) and about one litre of water were placed in a flask and left at room temperature for 24 hr. The permanganate was completely reduced. The manganese dioxide was filtered off and washed with water. When the filtrate was neutralized, a white product separated. It was filtered off and washed with water until free from potassium chloride. It was very soluble in methyl alcohol, acetic acid and most organic solvents. However, it was possible to recrystallize it from a mixture of one part of methyl alcohol and four parts of water. Dried over anhydrous calcium chloride, it melted at 120° C. The product obtained is a hydrate containing one molecule of water. When it melts, the water is set free and, at a higher temperature, solidification again takes place.

The product was characterized by analysis after it had been crystallized from dilute alcohol and dried over anhydrous calcium chloride. The silver salt was prepared by the usual method.

β-[3-Nitrophenyl]-β-[(10)-oxy-anthron-(9)-yl-(10)]-propionic acid is much less stable than the β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10]-propionic acid. It is readily decomposed by a boiling solution of potassium hydroxide (10%), yielding anthraquinone.

Heated with concentrated sulphuric acid at 150° C., the oxy-acid gave rise to the lactone and oxy-benzanthrone. Five grams was treated with 50 cc. of concentrated sulphuric acid and heated at 150° C. for five minutes. The mixture was poured on ice. The precipitate formed was filtered off, dried

and a portion was dissolved in hot chloroform. On cooling the solution, a fairly large quantity of red crystals separated. They were filtered off and identified as Bz-1′-oxy-Bz-3′(3-nitrophenyl)-1-9-benzanthrone by a mixed melting point determination.

The solution in chloroform was evaporated to dryness and the residue, purified by crystallization from acetic acid, was identified as the lactone by a mixed melting point determination. Calcd. for $C_{23}H_{17}O_6N \cdot H_2O$: C, 65.53; H, 4.54%. Found: C, 64.21; H, 4.55%. Calcd. for $C_{23}H_{16}O_6NAg$: Ag, 21.11%. Found: Ag, 21.21%.

(b) Preparation by hydrolysis of the lactone. The lactone (1 gm.) was treated with 0.15 gm. of potassium hydroxide in 5 cc. of alcohol (95%). The mixture was boiled for 15 min., cooled and diluted to 100 cc. with water. It was then filtered with suction. The dried residue (0.3 gm.) was identified as the starting material. The filtrate was acidified with hydrochloric acid (10%), and the precipitated acid was filtered off, washed with water and dried over anhydrous calcium chloride (0.6 gm.). It was dissolved in a hot saturated solution of sodium bicarbonate. After cooling, the solution was filtered and acidified again. This procedure was repeated twice. The acid was well washed with water and dried over anhydrous calcium chloride. It had the same properties as the acid prepared by the other method—it was very soluble in most of the organic solvents; it could be crystallized from a mixture of one part of alcohol to four parts of water; it was readily decomposed by a solution of potassium hydroxide (10%), yielding anthraquinone. When it was treated with concentrated sulphuric acid, Bz-1′-oxy-Bz-3′(3-nitrophenyl)-1-9-benzanthrone was formed. Calcd. for $C_{23}H_{17}O_6N \cdot H_2O$: C, 65.53; H, 4.54%. Found: C, 65.12; H, 4.21%.

References

1. BALLY, O. Ber. 38 : 194-196. 1905.
2. GAGNON, P. E. and GRAVEL, L. Can. J. Research, 8 : 600-615. 1933.
3. GRAVEL, L. Naturaliste canadien, 60 (3) 4 : 221-255. 1933.
4. KNOEVENAGEL, E. Ber. 31 : 2585-2619. 1898.
5. KÖTZ, A. J. prakt. Chem. 75 : 433-516. 1907.
6. MEYER, R. Ann. 219 : 234-306. 1883.
7. MILLER, W. v. and ROHDE. Ber. 23 : 1887-1902. 1890.

A PORTABLE GEIGER-MÜLLER TUBE COUNTER AS A DETECTOR FOR RADIOACTIVE ORES[1]

By G. M. Shrum[2] and Ronald Smith[3]

Abstract

A portable Geiger-Müller tube counter constructed for use as a geophysical instrument for locating radium-bearing ore bodies is described. Its chief characteristics are stability and low operating voltage, combined with great sensitivity to gamma radiation. The weight, using standard batteries, is about 55 lb. It is self-contained and can be used in rough country and in wet weather. Other uses suggested are in connection with natural gas wells, radium springs, mining operations, and for the location of lost radium needles and preparations used in hospitals.

Introduction

As the rich radium-bearing ore deposits in the Great Bear Lake district were discovered as outcroppings without the aid of scientific instruments, it seems reasonable to assume that other deposits might be discovered if suitable prospecting devices for penetrating the glacial overburden were obtainable. The gold-leaf electroscope is about the only instrument which has hitherto been available for this purpose. Although it is simple and portable, it is a very unsatisfactory instrument for field work, because of its fragility, the necessity for keeping it free from moisture, and the time required for taking readings. Any instrument developed to replace the electroscope should meet the following requirements: (*a*) It should be rugged enough to withstand field conditions; (*b*) the sensitivity should not be less than that of a laboratory gamma ray electroscope; (*c*) the time required for readings should be a minimum; (*d*) the instrument should be so simple in operation that it could be used effectively by persons with no special training in physics. By utilizing the principle of the Geiger-Müller counting tube an instrument has been developed which under actual test has fulfilled these requirements.

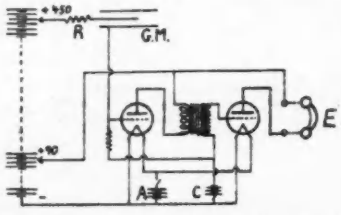


Fig. 1. *Diagram showing the simple amplifying circuit used with the portable Geiger-Müller counting tube.*

The Geiger-Müller Counting Tube

The electron counting tube developed by Geiger and Müller (5) and others (3, 6, 7, 11) for detecting gamma rays is well known. Fig. 1 is a diagram of the arrangement usually adopted for the operation of these counting tubes. The tube *G.M.* has as essential parts concentric electrodes placed so that the cylindrical brass cathode surrounds the fine tungsten wire anode.

[1] *Manuscript received July 30, 1934.*

Contribution from the Physics Laboratory, University of British Columbia, Vancouver, Canada.

[2] *Associate Professor of Physics, University of British Columbia.*

[3] *Assistant in Physics, University of British Columbia.*

It is filled with a gas at 6 to 8 cm. pressure. A battery maintains the cylinder at a high negative potential with respect to the wire. R, a resistance of the order of 10^9 ohms, is placed in series with the tube. A two- or three-stage vacuum-tube amplifier with ear phones, E, or loud speaker is used to detect current surges in the circuit. When the potential across the tube is 1000-1500 volts, discharges occur. When amplified, these produce sharp clicks in the receiving set. The frequency of these discharges is greatly increased if a small quantity of radium is placed close to the tube. The residual or accidental discharges in the absence of radium are due to cosmic rays and stray gamma rays from the earth, the walls of the room or the parts of the apparatus. There is a definite minimum operating potential which varies with the dimensions of the wire and the surrounding metal cylinder, and on the nature and pressure of the gas in the tube. Above this minimum or threshold voltage there is a range, the breadth of which varies with the tube, in which the count per minute is fairly constant. This range (voltage plateau) is one in which the tube must be operated. At still higher voltages spurious discharges take place, the number increasing with the applied voltage.

The extreme sensitivity of these tubes to gamma radiation makes them very useful as detectors of radioactive substances. However, for field work a constant d.-c. potential of 1000-1500 volts offers many difficulties. Therefore it was necessary to develop a tube of great stability but with a lower operating potential.

A Low Operating Potential

In the development of a tube with a low operating voltage it was necessary to consider the three main factors which determine the threshold potential. They are: (1) diameter of the outside metal cylinder, (2) pressure of the gas in the tube, and (3) nature of the gas.

The variation of the threshold potential, V, with the diameter, b, of the metal cylinder is given in the formula (2)

$$X = \frac{2V}{a \log \frac{b}{a}},$$

where X, the electric field at the surface of the wire, remains constant for a constant wire diameter a; that is, the less b is, the lower will be V. However, the lowering of b is accompanied by a corresponding decrease of the effective area of the tube, thereby decreasing the sensitivity. In consideration of these factors a value for b of $\frac{1}{2}$ to $\frac{5}{8}$ in. was chosen. The voltage varies very little with the radius of the wire, fine tungsten or manganin wire being used throughout.

It has been shown (2) that the threshold potential of a Geiger-Müller tube changes with gas pressure in the same way as does the sparking potential of the gas used. Consequently it would be expected that a considerable decrease in potential should be obtained by lowering the pressure of the gas.

By this means the voltage may be reduced to 600 or 700 volts for air at 1 to 10 mm. pressure. However, it was found that at this low pressure spurious discharges were produced at voltages only a little above the threshold; thus it is evident that such tubes would be useless for work requiring accurate measurement. In addition the sensitivity (count per minute) was very appreciably decreased at these pressures, as Geiger and Müller have already pointed out (6).

The third variable, the nature of the gas, held the greatest promise of giving advantageous results. Gases other than air have been used successfully in these tubes, it being found that each gas requires in general a different operating voltage. The lowest potential reported by Geiger and Müller for any gas investigated by them was that for argon (6). Bosch and Klumb (1) and Schulze (10) have shown, however, that the inert gases are undesirable for use in these tubes. Using pure neon and helium they found that a continuous discharge took place at voltages slightly above the threshold. These results for helium and neon were confirmed by the writers and a similar effect with argon was observed. It was found, however, that when very small quantities of air were added to the argon, the continuous discharging ceased and the tube functioned normally. The threshold potential of a tube containing argon with traces of air was 360 volts at 1 cm. pressure. The reliable voltage range was about 50 volts—a value which compared favorably with that for the regular high voltage tubes.

When these argon tubes were sealed off, their characteristics sometimes changed over a period of time. When the brass was too well baked out before admitting the gas mixture the impurity seemed to be re-absorbed by the metal, and consequently the tube developed a tendency to discharge continuously. On the other hand, if no de-gassing was done before filling, the threshold voltage would gradually rise, apparently owing to a change in the composition of the gas mixture due to the emission of adsorbed gases. To obtain a condition under which the gas mixture would remain constant indefinitely, the tube was only partially de-gassed. It was then filled to the proper pressure with the gas mixture. It was not always possible to gauge accurately the amount of air admitted. When it was too small, sufficient additional gas could usually be obtained by heating the walls of the tube. From 7 to 10 mm. pressure of argon was found most satisfactory. This gave an operating potential of from 350 to 450 volts. Neon was found to behave in much the same way as argon, although under the same conditions the neon tubes showed a slightly lower threshold voltage.

The Complete Portable Outfit

A self-contained portable outfit comprising a tube and its auxiliary equipment was constructed.

The design of the tube adopted for field work is shown in Fig. 2. A tube sealed in glass was used to obviate the leakage and insulation difficulties encountered with metal–ebonite tubes. The outer electrode *B*, brass tubing

$\frac{5}{8}$ in. in diameter and from 1 to 6 in. long, is held in place by a copper wire *A* and the tungsten wire *T* sealed into one end of the Pyrex glass covering *D*. The central electrode, *M*, a fine tungsten or manganin wire, is suspended at one end from a glass hook, *G*, fused on to the glass wall, and at the other by a second tungsten wire sealed into the outer case. The final sealing, at *S*, is made at one end, eliminating all side projections and allowing the tube to be conveniently handled or packed. One end, T^1, is immersed in paraffin wax contained in another tube of slightly larger diameter; the series resistance, *R*, is also embedded in the paraffin. This outer covering acts not only as a protection against breakage but also prevents any electrical leakage at the terminal T^1, an important factor because fluctuations in the leakage current over the insulation are amplified and recorded as impulses. The whole is enclosed in a cylindrical wooden case, which serves as a protection against mechanical shock and also excludes the light, thereby preventing the photo-electric effects described by Rajewski (9) and Locher (8). The wooden case is mounted on the end of a hollow handle, through which the connection with the remainder of the apparatus is made by ordinary rubber-covered wire.

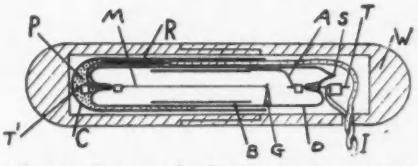


FIG. 2. *Diagram showing the mounting used for the portable Geiger-Müller tube. The paraffin wax,* P, *and glass tube,* C, *provide electrical insulation, while the wooden case,* W, *prevents breakage. The series resistance,* R, *is embedded in paraffin wax.*

The auxiliary equipment consists of a set of ten 45-volt batteries, a two-stage audio amplifier and a pair of ear-phones. The electrical arrangement is shown in Fig. 1, the plate voltage for the amplifier being tapped from the batteries used to supply the Geiger-Müller tube. The batteries and amplifier are placed in a waterproof case mounted on a standard pack-board, the whole weighing about 55 lb. This weight could be reduced by about 20 lb. by using specially designed batteries of light weight.

Sensitivity Tests

As with all small Geiger-Müller tubes, the number of residual or accidental discharges per minute due to cosmic rays and radioactive impurities undergoes a statistical fluctuation. One tube gave the following one-minute readings for 20 consecutive minutes: 10, 12, 11, 8, 10, 13, 9, 10, 9, 13, 6, 11, 14, 9, 9, 13, 7, 10, 10, 10. For longer periods, however, the fluctuations are much smaller, as the following five-minute readings obtained from the same tube show: 51, 54, 49, 50. Thus it may be seen that by taking five-minute readings an increase of five per minute in the count could be definitely detected. In case of doubt whether a high count is due to additional radioactive material in the neighborhood or to statistical fluctuation of the normal count, a longer reading period is required.

Laboratory Tests

Tests were made with radium and radium ore. A 53-gm. piece of pitchblende from Great Bear Lake containing 60% uranium oxide and about $\frac{8}{1000}$ mgm. of radium gave a count of 62 for five minutes when six feet from the detecting tube and 88 when three feet away. The zero count (no radium in the neighborhood) was 51 for a five-minute interval.

Eighty milligrams of radium in steel needles and enclosed in a small lead container was detected at a distance of 350 ft. taking only five-minute readings. Results of a test with this standard are shown in Table I. When these

TABLE I

RESULTS OF A TEST WITH 80 MGM. OF RADIUM IN STEEL NEEDLES IN A LEAD CONTAINER

Distance, ft.	100	150	200	250	300	350
Count*, 5 min.	265	139	99	82	64	55

* *Zero or normal count, 45.*

results are plotted with the count as ordinate and $\frac{1}{d^2}$ as abscissa, the inverse square law is obeyed within the limits of statistical error.

Field Tests

Field tests were carried out on Quadra Island in the Gulf of Georgia, from which samples of carnotite have been obtained. Fortunately a small section of this island had already been carefully surveyed by a party using a gold leaf electroscope, and the results were available for purposes of comparison. The electroscope gave some fairly high readings in certain areas and the survey indicated the possibility of commercial deposits of carnotite being found. Tests with the tube counter, however, showed that the only deposits were extremely small and localized. So far, subsequent exploration and development work on the property has confirmed the findings with this instrument.

Other Uses

It is well known that helium in varying proportions is a constituent of most natural gases. This helium is probably due to radioactive transformations in the neighboring rock structures. This suggests possibilities which could be investigated by lowering into the well the exploring tube of one of these instruments. It may be used also in the investigation of radium springs. And again in mining operations, one of these tubes could be lowered into a diamond drill hole, and thus be useful in locating any minerals which may be associated in some way with radioactive rocks.

Curtiss (4) has recently described a portable detector for locating small radium needles, etc. Judging from the various tests, it would appear that the instrument described here is probably 20 times as sensitive as that of Curtiss.

Acknowledgments

The authors wish to express their indebtedness to Dr. H. W. Riggs who kindly donated to the University a small quantity of radium, which has been most useful in these experiments; and to Dr. C. W. Prowd of St. Paul's Hospital, Vancouver, for the loan of radium needles.

References

1. BOSCH, C. and KLUMB, H. Naturwissenschaften, 18 : 1098. 1930.
2. COOKSEY, D. and HENDERSON, M. C. Phys. Rev. 41 : 392. 1932.
3. CURTISS, L. F. Bur. Standards J. Research, 5 : 115-123. 1930.
4. CURTISS, L. F. Bur. Standards J. Research, 12 : 379-382. 1934.
5. GEIGER, H. and MÜLLER, W. Physik. Z. 29 : 839-841. 1928.
6. GEIGER, H. and MÜLLER, W. Physik. Z. 30 : 489-493. 1929.
7. KNIEPKAMP, H. Physik. Z. 30 : 237-238. 1929.
8. LOCHER, G. L. Phys. Rev. 42 : 525-546. 1932.
9. RAJEWSKY, B. Physik. Z. 32 : 121-124. 1931.
10. SCHULZE, W. Z. Physik, 78 : 92-110. 1932.
11. VAN DEN AKKER, J. A. Rev. Sci. Instruments, 1 : 672-683. 1930.

EXPERIMENTAL PROBABILITY[1]

By A. L. Clark[2]

Abstract

The work described in this paper is a continuation of that published previously (1).

The observations have now been carried to 250,000. The results are in substantial agreement with those already published. The theoretical probability is 0.3554 ±0.0005, and the new experimental value is 0.35553. The value of π is not changed by the later results. The numbers of sequences of different lengths have been recorded and compared with the calculated values. The agreement here is not very good.

In a previous paper an experimental investigation of probability was described and the very close agreement between calculated and experimental values was shown (1).

The work described in this paper is a continuation of that already discussed and the number of events has been increased from 100,000 to 250,000.

The method employed in all of the work is briefly as follows. Steel balls are dropped at random on to a steel plate pierced with holes in regular array. Since in these experiments, the array is triangular (hexagonal), the probability that a ball will pass through a hole without contact with the plate is

$$P = \frac{2\pi (R-r)^2}{d^2 \sqrt{3}} , \tag{1}$$

where R and r are the radii of the holes and balls respectively and d is the distance between adjacent holes. There are certain corrections which must be applied to this value, and when these are applied the calculated value of the probability P is

$$P = 0.3554 \pm 0.0005. \tag{2}$$

The experimental probability is the ratio of the number of passages of the balls without contact with the plate, divided by the total number of balls dropped. From the 100,000 events of the earlier paper the value of the experimental probability was 0.35558. By a process of averaging or interpolation carried out on the graph of the results, the value 0.35556 was taken as the best value obtainable from the data.

From the later experiments using 250,000 events, the final value of P is 0.35555. When P is plotted by 10,000's, the value obtained from the graph is 0.35550. If P is plotted by 25,000's on a larger scale, the value of P is slightly higher, 0.35553. There is therefore still a little uncertainty about the last figure.

It was pointed out in the previous paper that, while the agreement between the calculated and observed values of P is very good, it is really better than might be expected from the accuracy with which the various measurements

[1] *Manuscript received July 23, 1934.*
Contribution from the Department of Physics, Queen's University, Kingston, Ontario, Canada.

[2] *Dean of Applied Science, Queen's University.*

of diameters and distances may be carried out. The newer work gives even closer agreement than was obtained before and it may be concluded, as was suggested, that the errors in the measurements of diameters and centre distances have neutralized each other to some extent.

Table I shows the values of the experimental probability and the fluctu-

TABLE I

TABULATED RESULTS

5,000's		10,000's		25,000's		50,000's		100,000's		To date	
P	δ	*P*	δ	*P*	δ	*P*	δ	*P*	δ	*P*	δ
.3516	−.0039									.3516	.0039
.3554	−.0001	.3535	−.0020							.3535	.0001
.3596	+.0041									.3555	.0000
.3516	−.0039	.3556	+.0001							.3546	−.0009
.3548	−.0007			.35460	−.00093					.3546	−.0009
.3614	+.0059	.3581	+.0026							.3557	+.0002
.3596	+.0041									.3563	+.0008
.3546	−.0009	.3571	+.0016							.3561	+.0006
.3456	+.0001									.3549	−.0006
.3654	+.0099	.3555	.0000	.35732	+.00179	.35596	+.00043*			.35596	+.00043
.3508	−.0047									.35555	+.00002
.3592	+.0037	.3550	−.0005							.35580	+.00027
.3512	−.0043									.35545	−.00008
.3538	−.0017	.3525	−.0030							.35533	−.00020
.3548	−.0007			.35396	−.00157					.35529	−.00024
.3574	+.0019	.3561	−.0006							.35543	−.00010
.3580	+.0025									.35558	+.00005
.3578	+.0023	.3579	+.0024							.35570	+.00017
.3548	−.0007									.35565	+.00012
.3542	−.0013	.3545	−.0010	.35644	+.00091	.35520	−.00033	.35558	+.00005	.35558	+.00005
.3622	+.0067									.35590	+.00037
.3598	+.0043	.3610	+.0055*							.35608	+.00105
.3440	−.0115									.35555	+.00002
.3564	+.0009	.3502	−.0053							.35558	+.00005
.3574	+.0019			.35596	+.00043					.35566	+.00013
.3460	−.0095	.3517	−.0038							.35528	−.00025
.3632	+.0077									.35558	+.00005
.3532	−.0023	.3582	+.0027							.35550	−.00003
.3632	+.0077									.35576	+.00023
.3458	−.0097	.3545	−.0010	.35428	−.00125	.35512	−.00041			.35543	−.00010
.3636	+.0081									.35569	+.00016
.3532	−.0023	.3584	+.0029							.35561	+.00008
.3580	+.0025									.35568	+.00015
.3472	−.0083	.3526	−.0029							.35544	−.00009
.3702	+.0147*			.35844	+.00291*					.35586	+.00033
.3506	−.0049	.3604	+.0049							.35571	+.00018
.3468	−.0087									.35547	−.00006
.3580	+.0025	.3524	−.0031							.35554	+.00001
.3574	+.0019									.35558	+.00005
.3518	−.0037	.3546	−.0009	.35292	−.00261	.35568	+.00015	.35540	−.00013*	.35550	−.00003
.3560	+.0005									.35550	−.00003
.3632	+.0077	.3596	+.0041							.35569	+.00016
.3488	−.0067									.35553	.00000
.3540	−.0015	.3514	−.0041							.35549	−.00004
.3568	+.0013			.35576	+.00023					.35552	−.00001
.3582	+.0027	.3575	+.0020							.35558	+.00005
.3530	−.0025									.35552	−.00001
.3498	−.0057	.3514	−.0041							.35540	−.00013
.3634	+.0079									.35557	+.00004
.3548	−.0007	.3591	+.0036	.35584	+.00031	.35580	+.00027			.35555	+.00002

NOTE:—*P is the experimental probability; δ is the fluctuation or the departure from the mean.*

ations about the mean, for series of different lengths. Since each page of the record book contains 1000 observations, it has been convenient to deal with the results by these 1000's. It might be, of course, that by selecting a different place, such as the middle of a page for the starting point of a series, slightly different and perhaps more interesting results might appear, but it is deemed better to deal with the observations in the more natural groupings which in a very real sense are random groupings.

These fluctuations are interesting and show clearly that, as the series length increases, the relative fluctuations decrease. There are exceptions, as it might be supposed would be the case. The word "expect" is hardly proper when discussing probability. Strictly speaking, one has no right to expect anything in probability experiments. One may predict (successfully or otherwise) but since anything may happen, expectation is barred.

The maximum values of the fluctuations are asterisked. These bring out very clearly what was pointed out in the earlier paper, *viz.*, the maximum relative fluctuations decrease very rapidly as the series length increases. One may not say that this will be the case always, since the improbable may happen at any time and upset all predictions, a point that can hardly be overemphasized.

As was done with the earlier results, the values of P by 100's for the entire series were noted and the number of times that each value appeared was plotted as a function of the value. Fig. 1 shows the result, and the resemblance to the ordinary probability curve is clearly seen. The fluctuations for this series length are seen as the departures of the values of P from the mean value which is indicated by the dotted line.

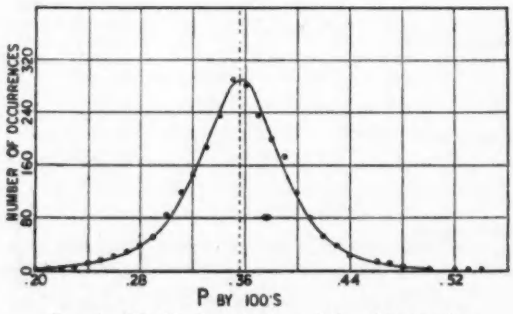


FIG. 1. *Distribution of values of P and fluctuations.*

The Value of π

As shown in the first paper, if P is known by experiment, π may be calculated from Equation (1). The value of π calculated in this manner for the first 100,000 events was 3.143 ± 0.005. The value of P for the second 100,000 is 0.35540, which is the *calculated value.* Hence if this value of P is used, the value of π should be 3.1416, the number used in calculating P. If Equation (1) may be regarded as a functional relation between P and π, π is proportional to P, and questions as to significant figures in the reverse calculation do not enter. The terminal value of P, 0.35555, and the averaged value 0.35553, differ so little from the value 0.35556 used in the earlier calculations, that the value of π is not affected sufficiently to show in the number of decimal places allowable.

Sequences

The numbers of sequences, or runs of 2, 3, 4, 5, etc., are calculated from

$$N = P^k n ,$$

where N is the number of sequences of length k, in a series of length n. Table II shows the calculated and observed values of N for values of k obtained from the experiment. The value of P is taken as 0.3555 which is near enough for this calculation. The agreement between the calculated and the observed values is on the whole not as good as shown in the previous paper for a series of 100,000. It is not surprising however that, even for long sequences where the number of occurrences is small, the agreement is not very good. That the earlier work showed better agreement must be regarded as fortuitous.

TABLE II

SEQUENCES

	By 50,000's						By 100,000's			For 250,000	
	Calc.	Observed					Calcd.	Observed		Calcd.	Obs.
		1st	2nd	3rd	4th	5th					
1's	17775	17798	17760	17756	17784	17790	35550	35558	35540	88875	88888
2's	6319	6501	5864	6236	3246	6022	12638	12259	9482	31595	29883
3's	2246	2483	2178	2336	1859	2226	4693	4655	4195	11232	11076
4's	799	834	655	720	503	675	1597	1489	1223	3993	3387
5's	284	346	220	253	157	230	568	572	410	1420	1207
6's	101	140	51	62	20	42	202	191	82	505	315
7's	36	66	8	15	3	10	72	74	18	179	102
8's	13	31	2	5	0	1	26	33	5	64	39
9's	4	17	0	0	0	0	9	17	0	22	17
10's	1	9	0	0	0	0	3	9	0	8	9
11's	.6	5	0	0	0	0	1	5	0	3	5
12's	.2	3	0	0	0	0	.4	3	0	1	3
13's	.07	1	0	0	0	0	.1	1	0	.3	1

The sequences are shown for series lengths of 50,000, 100,000 and for the entire series of 250,000.

Discussion

It was stated rather positively in the earlier paper that 0.35556 might be taken as a very close approximation to the true value of P. In the light of the subsequent work this statement must be modified a little. The value of P rose to 0.35608 at 110,000 and fell to 0.35498 at 128,000. Thus even after a fairly long series has been completed and the conclusion has been reached that there will be no more fluctuation of a given amount, a few very low or very high values for a few thousands throw the value of P outside the limits deemed assignable. The third figure in the value of P has remained unchanged

however since the end of the 128th thousand and it is improbable that it would ever change again. The fourth figure, however, cannot safely be said to have reached a fixed value even for 250,000, although it is extremely probable that its value too will soon become constant. This fourth figure fluctuated up and down even in the fifth 50,000. Throughout the entire experiment, the observers were worried, first, lest the results were getting too large, and a few days later, lest they were getting too small. Several 1000's might be large, then several 1000's small, or again they might oscillate up and down. This is, of course, precisely what should happen, but during the course of the observations the feeling grows, one way and then the other, that something is wrong with the apparatus. Eventually, and no doubt not much further on, the fourth figure would become fixed, and then the fifth, and perhaps the sixth, if we had the courage and time to carry the series far enough. Of course one must be prepared for the improbable and the third figure or indeed the second might change.

There are slight grounds for suspicion that the areas of the holes in the portion of the plate more remote from the edges have been decreased slightly by the continued impact of the balls on to the plate. This reduction in no case is over 0.0001 in., and in most cases is much less. The effect on P is difficult to calculate without such a detailed study of the shapes and sizes of all of the holes as would involve an unjustifiable expenditure of time. It is safe to say, however, that such a distortion could not affect the value of P by more than 0.0002, which is well within the limits of error set. It may be, however, that the slight decrease in the experimental probability shown in the results is due to a slight decrease in available area of the holes.

The correction factor, 0.9983, applied in the earlier work, should be increased a little (but only a little) by improvement in the verticality of fall. It was noticed that whenever a fresh lot of balls was used, the departure from the vertical was always less. After careful investigation, it was found that if the balls were carefully cleaned and then given a very slight coating of grease, the paths were more nearly vertical and fewer balls departed from the vertical by more than a millimetre. The departure from the vertical is evidently caused by a slight rotary motion as the ball falls through the tube. Neither the actual direction nor the amount is predictable since both depend on the last contact of the ball with the dropping tube. The grease seems to cause a slight adhesion to the wall of the tube and much of the rotary motion is stopped. The balls always have a coating of grease when received from the makers.

The two effects mentioned in the last two paragraphs compensate each other to a considerable extent and the calculated value, 0.3554 ± 0.0005, need not be changed.

A point which came up through an unpublished criticism of the former paper is the appearance of the sequences of 12 and 13 in the first 50,000 trials. It was maintained that a 13 would appear once in 1,000,000 events, and therefore its appearance in the 49th thousand is an indication that

randomness had not been attained. In reply it is only necessary to ask in what 1000 could the appearance of a sequence of 13 be admitted. The criticism seems to imply that all 1000's are not alike and that perhaps after 1000 thousands have been run off, a peculiar one will appear in which a 13 might enter. That far-distant thousand seems to have magical properties. The current 1000 however is the only one in which an extraordinary or improbable phenomenon may appear and the far-distant one never arrives. We always have to do with the present.

Again, one has only to point to the remarkable agreement between experimental and calculated values of P to remove any doubt as to randomness. We have to conclude that the early appearance of long sequences was exactly what might occur. Finally, the failure of some of the experimental sequence values to agree with those calculated (Table II) must be explained in precisely the same way. We must be prepared to accept the appearance of the improbable or the non-appearance of the probable in limited probability experiments.

It may be argued that the early appearance of a long sequence might be due to a momentary relaxation of attention during the supposed sequence, with the result that a contact was not heard. Such a thing is possible but highly improbable. Neither will lack of experience do as an explanation, since 20,000 events were run off before the record was begun. Finally there is the possibility of a faint sound due to very light contact of the ball with the plate, so light as to escape notice*. This is possible, but the difference in the paths of the ball between hit and miss is so small as to make such an explanation of little value. In order to test this point the following was carried out. One of the balls used in the experiment was soldered to the top of the plate and a micrometer screw was arranged vertically on a swivel so that it might be swung on a vertical axis. The end of the micrometer screw therefore passed over the ball. Then by turning the micrometer screw, it could be made to approach the ball more and more closely, while the screw was constantly rotated on the swivel. Eventually the end of the screw touched the ball. The microphone gave substantially the same sound as when in the experiments a ball made glancing contact with the surface of one of the holes. By adjustment of the micrometer, the sound could be made heavy or light. It was found that the amount of motion of the screw to change from free passage to contact was very small indeed—*i.e.*, is of the order of 1/10,000 the radius of the hole—perhaps less. For a ball to make a contact which might escape detection its centre must lie in an annular ring whose area is so small that the probability of such an occurrence is approximately the same as the probability that a run of 13 might occur.

The experiment as a whole clearly indicates several things. (i) If randomness obtains, the values of the experimental and calculated probability agree

**A statement in the earlier paper may be a bit misleading. It was stated that there is no gradation of sound for grazing contacts. The character of the sound does not change but it is light or heavy according to the violence of the shock. There seem to be no "threshold" cases where one is undecided about the contact.*

very closely if the series is sufficiently extended. In an experiment where measurements of distance or length are involved, the experimental value gives a more accurate determination of the true probability than may be calculated from measurement, provided the series is long enough. (ii) Anything possible may happen at any time and extremely improbable phenomena may occur even in a moderately short series. (iii) The highly probable may fail to occur in a finite series. (iv) It is not permissible to draw conclusions from a few events and the practice of assigning probable values from a few observations may not be justifiable in some cases.

Acknowledgments

The author acknowledges his indebtedness to Mr. E. Harris, Curator in the Department of Physics, for his untiring zeal in carrying on the experimental work. The author also wishes to thank a group of Honor students for their assistance.

Reference

1. CLARK, A. L. Can. J. Research, 9 : 402-414. 1933.

REVIEWS AND NOTES

The Specific Heats and Densities of Some Hydrophobic Sols

Zwicky (8) has explained the anomalous specific heats of some electrolytes as a result of the strong electric field which exists in the neighborhood of the ions. The field polarizes the water around the ions and produces a hydrostatic pressure of many thousand atmospheres. Bridgman (1) has shown that the specific heat of water decreases with increase of pressure, and using his values Zwicky has calculated that this effect is large enough to account for the observed decrease in the specific heats of the solutions.

Müller (3) has suggested that this effect must also be present in colloidal solutions, and that it is large enough to explain the stability of sols which, on the basis of other theoretical considerations, contain particles too large to remain in suspension. Ostwald (4) and others have pointed out that this compression of the liquid surrounding the colloid particles should lead to an increase in the density of the solution.

Careful measurements were made of the specific heats of gold and copper hydrosols using the method of cooling. The specific heats of these sols (concentration about 40 mgm. in 100 cc. of solution) were found not to differ from that of water by more than one part in 400 over a temperature range from 25 to 30° C.

The densities of gold and copper hydrosols were measured by the method outlined by Washburn (6). In each of the samples tested the density was slightly less (not more than 4 parts in 100,000) than that calculated from the weight of the residue when the sol was evaporated. Paine's (5) experimental values for the densities of copper hydrosols (obtained in a study of their chemical constitution) are in agreement with calculated values to within 1 part in 100,000.

Free energy changes accompanying the formation of a colloidal solution were calculated by both Zwicky's and Webb's (7) methods. Even assuming particle charge of 1000 electrons and a concentration of 10^{13} particles per cc. (10^{-6} cm. radius), the compression effect is only about 10^{-6} calories per cc., and the polarization about 10^{-7} calories per cc. In accordance with these results measurable changes in specific heat and density are not to be expected.

The writers wish to express their sincere appreciation to Prof. E. F. Burton, of the Department of Physics, University of Toronto, for his helpful suggestions and advice throughout the investigation.

References

1. BRIDGMAN, P. W. Proc. Am. Acad. 48 : 310-362. 1913.

2. EVJEN, H. M. and ZWICKY, F. Phys. Rev. 33 : 861-868. 1929.

3. MÜLLER, Stability of colloids and the theory of rapid coagulation. Symposia on quantitive biology. Vol. I. 1933.

4. OSTWALD, W. A handbook of colloid chemistry. P. Blakiston's Sons and Co. Philadelphia. 1919.

5. PAINE, H. H. and SHEARSMITH, H. W. S. African J. Sci. 30 : 127-131. 1933.

6. WASHBURN, E. W., SMITH, E. R. and FRANDSEN, M. Bur. Standards J. Research, 11 : 453-462. 1933.

7. WEBB, T. J. Trans. Am. Electrochem. Soc. 51. (Preprint) 9 pp. 1927.

8. ZWICKY, F. Physik. Z. 27 : 271-286. 1926.

MAY ANNETTS

HAMILTON SIMS

THE MCLENNAN LABORATORY,
UNIVERSITY OF TORONTO,
TORONTO, ONT., CANADA.

RECEIVED JULY 12, 1934.